Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Identification of pinocembrin from Romanian propolis as an effective antifungal agent against diverse phytopathogenic fungi.

Florent Rouvier
Emmanuel wafo
brunel jean michel
brunel.jm@yahoo.fr

Faculté de Pharmacie de Marseille: Aix-Marseille Universite Faculte de Pharmacie
https://orcid.org/0000-0002-9355-8980

Research Article

Keywords: Propolis, Fungi, F. oxysporum, B. cinerea, Z. tritici, Antifungal activity, Pinocembrin

Posted Date: July 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10202374/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Because fungal diseases are among the major constraints affecting modern agriculture, there is an urgent need to identify new, effective, and environmentally sustainable alternatives for crop protection. As part of our ongoing studies on propolis, we investigated the potential of natural bee-collected compounds to act against phytopathogenic fungi. *In vitro* evidence suggests that pinocembrin isolated from Romanian propolis exhibits antifungal activity and may effectively control the growth of several phytopathogenic fungi, including *F. oxysporum*, *B. cinerea*, and *Z. tritici*, thereby supporting the phyto-pharmaceutical potential of propolis as an ecological alternative for crop management in sustainable agriculture.

1. Introduction

Fungal diseases are among the major constraints affecting modern agriculture. They reduce yield and quality in many economically important crops, including cereals such as wheat and maize, and horticultural crops such as potatoes and tomatoes. According to the FAO, plant pests and diseases are responsible for losses of up to 40% of global crop production each year, creating major challenges for food security, trade, and farmer income [1]. The control of phytopathogenic fungi remains strongly dependent on resistant crop varieties, agronomic practices, and chemical fungicides. However, the repeated use of the same active ingredients has contributed to the emergence of resistant fungal populations, reducing the efficacy of available treatments. This is a growing concern for many crop pathosystems, including potato, tomato and especially wheat diseases caused by highly adaptable pathogens [2–3]. A relevant example is *Zymoseptoria tritici*, previously known as *Septoria tritici* is considered one of the most important threats to wheat production worldwide [4–7]. *Z. tritici* is also of strong industrial interest because it has developed reduced sensitivity or resistance to several major fungicide classes, including azoles and strobilurins. This makes it a useful biological model for studying fungicide resistance and for evaluating new crop-protection strategies[8–9].

In this context, there is an urgent need to develop new methodologies for the discovery, screening, and validation of antifungal compounds. Innovative approaches may include high-throughput bioassays, molecular and genomic tools, phenotypic screening, natural-product discovery, biocontrol strategies, and integrated disease-management systems. The objective is not only to identify new active molecules, but also to design sustainable products with novel modes of action, lower environmental impact, and reduced risk of resistance development. Therefore, research on phytopathogenic fungi such as *F. oxysporum*, *B. cinerea* and *Z. tritici* is essential for supporting the development of next-generation solutions for crop protection.

In the continuation of our studies on the valorization of propolis, we investigated the potential use of this biomass as a source of natural bee-collected molecules with activity against phytopathogenic fungi.

2. Results and discussion

Propolis which is a resinous substance produced by honeybees has been used in traditional medicine for millennia. The term derives from ancient Greek, combining *pro* meaning “in front of” and *polis* meaning “city”, and is commonly interpreted as “defense of the city” [10]. This etymology reflects the biological function of propolis in the hive, where bees use it to seal cracks, reinforce hive structure, and protect the colony against fungi, bacteria, and other external threats [11–13]. The chemical composition of propolis is highly variable and depends on honeybee species, botanical source, geographic origin, harvesting season, and climatic conditions. Therefore, propolis should not be considered a single chemically uniform material, but rather a family of natural products whose biological activities are strongly influenced by the surrounding flora. This variability is a key factor when interpreting pharmacological or antimicrobial studies, since different propolis types may not exhibit the same efficacy against a given pathology or microbial target. In our previous work, we investigated propolis samples collected in Rwanda, a region characterized by exceptional biodiversity, which led to the identification of two compounds with activity against *Cutibacterium acnes*, the bacterium implicated in acne [14–15].This ecological context may favor the presence of distinctive plant-derived metabolites in local propolis. As a first step, we tested a selection of propolis samples from different regions of the world against both *F. oxysporum* and *B. cinerea*, which are readily cultivated in our laboratory, to identify the most promising samples for further investigation. Among all the propolis samples tested, collected from various regions of the world, including geographically close areas, only two samples, designated **OFAP147** and **OFAP148** from Romania exhibited strong antifungal activity against *F. oxysporum*. As shown in Table 1, these samples completely inhibited fungal growth, resulting in a growth rate of 0%. In contrast, the growth of *Botrytis cinerea* was inhibited by only 31%.

Table 1 Antifungal activities of propolis samples collected from various regions worldwide against *F. oxysporum* and *B. cinerea*

Encouraged by these promising results, particularly, we sought to further investigate the **OFAP147** extract and identify the compound or compounds responsible for the observed antifungal activity against *F. oxysporum*. For this purpose, 15 mL of the crude ethanolic extract of **OFAP147** was concentrated under vacuum to obtain 1.4 g of crude powder. This material was then subjected to flash chromatography using a solvent gradient from non-polar to polar conditions, starting with petroleum ether, followed by ethyl acetate and methanol.

This fractionation yielded 53 fractions of 15 mL each, which were subsequently pooled and concentrated into six main fractions, designated **147 F-A** to **147 F-F**. After vacuum concentration, the corresponding masses were 0, 639, 435, 92, 53, and 560 mg, respectively.

The antifungal activity of these fractions was then evaluated. Growth inhibition assays were performed against two fungal strains, *F. oxysporum* (Fig. 1A) and *B. cinerea* (Fig. 1B), using a final concentration of 200 µg/mL of each hydroalcoholic propolis fraction. Among the tested fractions, **147 F-B** showed the strongest antifungal activity against *F. oxysporum*, indicating that the active compounds responsible for

this activity were mainly, or possibly exclusively, present in this fraction. In contrast, no significant inhibition was observed against *B. cinerea* under the same conditions.

Based on these results, fraction **OFAP147-B** was subjected to a second fractionation step using a petroleum ether/ethyl acetate gradient over 20 minutes, ranging from 0% to 100% ethyl acetate. A total of 73 fractions of 15 mL each were collected and subsequently pooled into 10 subfractions, designated **P147-B_F1** to **P147-B_F10**. The antifungal activity of these subfractions was then assessed. Fraction **P147-B_F3** displayed strong activity against *F. oxysporum*, whereas only weak inhibitory activity was observed against *B. cinerea* (Fig. 1).

Thus, fraction **P147-B_F3** was subjected to GC–MS chemical profiling (Fig. 2), which revealed the presence of pinocembrin [16–17]. This compound was unambiguously identified by comparison of its mass fragmentation pattern with the NIST library and by using the NIST 23 GC Method/Retention Index Database, which provides retention indices and gas chromatographic conditions for more than 180,000 compounds. The identification was further supported by ^{1}H and ^{13}C NMR analyses, confirming a purity greater than 95% (Fig. 2). On the other hand, measurement of the optical rotation using a polarimeter gave a positive value, allowing us to conclude that the isolated product corresponds to (*S*)-pinocembrin.

OFAP147 and commercially available (*S*)-(+)-Pinocembrin were subsequently evaluated on agar medium at different concentrations against both *F. oxysporum* and *B. cinerea* (Fig. 3).

Both pinocembrin and OFAP147 inhibited the growth of *Fusarium oxysporum* at 100 µg/mL. **OFAP147** also caused a pronounced reduction in mycelial growth at 50 µg/mL, a concentration at which sporulation was completely suppressed. Under the same conditions, the growth of *Botrytis cinerea* was reduced but not fully inhibited. In contrast, sporulation was strongly impaired, especially following treatment with **OFAP147**, including at the lowest concentrations tested.

In parallel, a method for determining the concentration required to inhibit fungal growth in liquid Potato Dextrose Broth (PDB) medium was developed leading to reproducible results against both fungal strains (Table 2).

Table 2
Antifungal activities of **OFAP147**, **P147-B_F3** and **(S)-(+)-Pinocembrin** against *F. oxysporum* and *B. cinerea* performing the tests in liquid media in triplicate.

Sample	**MIC (µg/mL)**	
	F. oxysporum	***B. cinerea***
OFAP147	63	31
P147-B_F3	63	31
(S)-(+)-Pinocembrin	63	63

The observed trend confirms the activity of pinocembrin against *F. oxysporum*, with growth inhibition detected at 100 µg/mL on agar medium and at 63 µg/mL in liquid medium. In contrast, inhibition of *B. cinerea* growth by pinocembrin was observed in liquid medium, whereas no clear inhibition was visible on solid medium. The differences in activity between solid and liquid media may be explained by differences in the availability of the active compound, as well as by the facilitated growth of spores on solid supports.

Subsequently, we were able to extend the spectrum of action of **OFAP147**, **P147-B_F3** and **(S)-(+)-Pinocembrin** on a panel of *Zymoseptoria tritici* strains. In this context four different well characterized strains [18] of *Z. tritici* were involved in this study (Table 3).

Table 3
Antifungal activities of **OFAP147** and **(S)-(+)-Pinocembrin** against four different strains of *Z. tritici* performing the tests in liquid media in triplicate.

Molecule	**Pino**	**P147**	**Pino**	**P147**	**Pino**	**P147**	**Pino**	**P147**
Strain	*Z. tritici* l93		*Z. tritici* IPO323		*Z. tritici* 3D7		*Z. tritici* IR01_48b	
MIC (µg/mL)	63	16	31	16	31	16	63	16

We were therefore able to demonstrate a strong correlation between the antifungal activity of the propolis extract against *Z. tritici* and, more specifically, that of pinocembrin itself. Indeed, a similar degree of activity was observed regardless of the strain tested, and comparable results were obtained with both pinocembrin and the OFAP147 propolis extract. Thus, Pinocembrin exhibited a similar level of antifungal activity against the four tested *Z. tritici* strains, with MIC values ranging from 31 to 63 µg/mL. This activity was comparable to that observed for the propolis extract **OFAP147**. To the best of our knowledge, this property has not previously been reported.

From a mechanistic point of view, literature provides several avenues for further reflection. The antifungal activity of (*S*)-(+)-pinocembrin has been investigated mainly *in vitro*, particularly against yeasts and postharvest molds. One of the clearest studies showed that pinocembrin isolated from propolis inhibited *Penicillium italicum* in a dose-dependent manner [19]. The proposed mechanism involved disruption of fungal energy metabolism, including inhibition of respiration during spore germination and mycelial growth, impairment of mitochondrial respiration, reduction of adenine nucleotide energy status, and membrane damage, as indicated by ionic leakage and protein loss. These results suggest that pinocembrin can interfere with fundamental physiological processes required for fungal growth and development. All these findings support our observations in both liquid medium and agar-based assays. However, the role of pinocembrin in propolis bioactivity should be interpreted cautiously. Although pinocembrin is a typical and often abundant propolis flavonoid, propolis activity is rarely attributable to

this compound alone. Chemometric studies have identified pinocembrin, chrysin, galangin, pinobanksin, pinobanksin-3-acetate, and related phenolics as markers associated with elevated antifungal potential [20–21]. This suggests that additive or synergistic interactions among propolis constituents are likely important.

3. Conclusion

Among the compounds frequently associated with the bioactivity of propolis, pinocembrin occupies a particularly important position. It is one of the characteristic flavonoids of temperate, poplar-type propolis. Because the chemical composition of propolis is strongly influenced by its botanical and geographical origin, the abundance of pinocembrin and related flavonoids can vary considerably among samples. This variability may partly explain the marked differences in antifungal activity observed among propolis extracts and particularly **OFAP147**. In the present study, we demonstrate for the first time the antifungal activity of pinocembrin at low MIC values against *F. oxysporum*, *B. cinerea*, and *Z. tritici*. These findings identify pinocembrin as a promising natural compound for the development of plant-protection strategies against major crop diseases. Further studies are currently underway to assess its safety profile, including its potential effects on soil quality, soil bacterial communities, beneficial insects, and other non-target organisms, as well as its broader ecotoxicological impact.

4. Materials and methods

4.1 Reagents

All solvents and reagents used were commercially available. Dichloromethane, methanol, ethyl acetate and petroleum ether were purchased from VWR and used without further purification. Column chromatography was performed on Macherey Nagel silica gel (70–230 mesh) using a COMBIFLASH device. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a 300 and 75 MHz Bruker AC 300 spectrometer, respectively (the usual abbreviations are used: s: singlet, d: doublet, t: triplet, q: quadruplet, m: multiplet). All chemical displacements are indicated in ppm. The analysis by mass spectroscopy was carried out by the laboratory of analysis of the faculty of pharmacy (University of Aix-Marseille).

Pinocembrin is Identified using gas 7890B chromatography coupled to a 5977A mass spectrometer (GC-MS Agilent Technologies), equipped with a capillary column (VF-5mS, CP8944, 30 m × 0.25 µm × 0.25 mm).Helium was used as carrier gas at a constant flow rate of 1 mL/min. 1 µL of the sample was injected in split mode at 250°C. The GC oven temperature program was as follows, temperature gradient: 40°C for 0 min, then 5°C increase up to 80°C for 1 min, then 5°C increase up to 100°C for 0 min, then 9°C increase up to 200°C for 30 min; analysis time: 90 min.). The ion source temperature was fixed at 200°C. the mass spectrometer operated in the electron impact ionization mode (70 eV; acquisition mode: full scan mode from 50 to 600 m/z)

4.2 Preparation of the hydroalcoholic extract of propolis OFAP147

Propolis **OFAP147** was collected in Romania (Dhnavatu de jos, Tulcea) as a black gum. Briefly, 10.1 g of **OFAP147** was extracted with 15 mL of ethanol (water:ethanol (40:60, v/v)) by using a Biotage initiator microwave apparatus operating for 10 minutes at 100°C and 3 bars. After centrifugation and filtration this lead to an ethanol extract which the propolis concentration was determined to be 93 mg/mL and was subsequently used for biological tests and flash chromatography (from non-polar to polar namely petroleum ether, ethyl acetate and methanol)

4.3 Fungal strains

The fungal strains tested were *Botrytis cinerea* ATCC 38602, *Fusarium oxysporum* DSM 841, *Zymoseptoria tritici* IPO323 (Netherlands), *Zymoseptoria tritici* 3D7 (Switzerland), *Zymoseptoria tritici* IR01_48b (Iran), *Zymoseptoria tritici* I93 (USA, Indiana).

4.4 Determination of antifungal activity on agar medium

Two fungal strains were selected for the antifungal activity assays on agar medium: *Botrytis cinerea* ATCC 38602 and *Fusarium oxysporum* DSM 841. These strains were chosen because they can be readily cultivated on Potato Dextrose Agar (PDA) and in Potato Dextrose Broth (PDB), allowing standardized growth and sporulation under laboratory conditions.

The initial screening method consisted of culturing spores of *B. cinerea* and *F. oxysporum* on solid medium in 24-well plates. For each test well, 10 µL of propolis extract at 10 mg/mL was added. The wells were then completed with 490 µL of molten PDA, previously cooled to approximately 40°C before dispensing. This procedure resulted in a final propolis extract concentration of 200 µg/mL in each test well.

Each plate included a solvent control (ethanol 1.2% final concentration in the well) to confirm that ethanol did not affect fungal viability. In addition, growth control and negative contamination control were systematically included in each assay. Each 24-well plate allowed the evaluation of 20 propolis samples against one fungal strain. After solidification of the medium, 5 µL of fungal spore suspension was deposited at the center of each well. The plates were then incubated at 30°C under saturated humidity conditions for 72 h.

Two parameters were assessed during the assay: the diameter of fungal colony growth and the presence or absence of sporulation in each well. Measurement was performed after 72 h of incubation.

The antifungal potential of each propolis extract was expressed as the percentage of fungal growth relative to the growth control and was calculated using the following formula:

$$\textbf{Growth percentage (\%)} = \frac{\textbf{Colony growth diameter in the test well}}{\textbf{Colony growth diameter in the positive growth control}} \textbf{ x100}$$

A value of 0% indicated complete inhibition of fungal growth by the propolis extract, whereas a value of 100% indicated the absence of antifungal activity.

The 72 h incubation period was selected to allow sufficient time for the colonies of both fungal species in the growth control wells to cover the well surface as extensively as possible and to ensure full development of sporulation. Under these experimental conditions, both fungal species reached maximal growth after 72 h, and sporulation was clearly visible at this time point. The sporulation patterns differed between the two species: *B. cinerea* produced dark green to black, powdery sporulation, whereas *F. oxysporum* developed a fluffy pink to violet sporulation.

4.4 Determination of antifungal activity by broth microdilution

The antifungal activity of the tested compounds was evaluated using a broth microdilution assay adapted from the Clinical and Laboratory Standards Institute (CLSI) guidelines. The method was based on the procedure used for bacterial minimum inhibitory concentration determination, with minor modifications specific to fungal growth conditions.

The assay was performed in sterile 96-well microplates with a final volume of 200 µL per well to improve reproducibility. Potato Dextrose Broth (PDB) was used as the culture medium. Pinocembrin and propolis extracts were prepared in 70% ethanol at a stock concentration of 10 mg/mL. Both samples were subjected to two-fold serial dilution. The first two dilutions were prepared in 70% ethanol, followed by subsequent dilutions in water. Working solutions ranging from 5000 to 80 µg/mL, corresponding to ethanol concentrations of 70% to 2.2%, were obtained. For each concentration, 10 µL was added to the wells of a row, followed by the addition of 190 µL of fungal inoculum. This resulted in final concentrations in the microplate wells ranging from 250 to 4 µg/mL, with final ethanol concentrations ranging from 3.5% to 0.1%. The fungal inoculum was prepared by diluting frozen spore suspensions 1:500 in PDB. After dispensing the test compounds, culture medium, and inoculum into the wells according to the plate layout, the microplates were covered with a Breath-Easy® sealing membrane (Ref. Z380059-1PAK, Merck SA, Lyon, France) to limit the risk of contamination during incubation while allowing gas exchange. The plates were incubated at 30°C under appropriate fungal growth conditions. As in bacterial microdilution assays, the minimum inhibitory concentration (MIC) was defined as the lowest concentration of the tested compound for which no visible turbidity was observed in the well. However, unlike assays performed on solid agar medium, this liquid microdilution method does not allow direct observation of sporulation.

All manipulations were carried out under a biological safety cabinet in a biosafety level 2 laboratory. The results were recorded on a dedicated, numbered experimental form to ensure traceability and were subsequently integrated into the digital propolis database.

Declarations

Conflicts of Interest:

The authors declare no conflict of interest

Funding:

This research received external funding from OFA (Observatoire Français d'Apidologie).

Author Contributions:

Conceptualization, F.R. and J.M.B.; Formal analysis, J.M.B.; Investigation, F.R., E.W. and J.M.B.; Methodology, F.R. and J.M.B.; Project administration, J.M.B.; Supervision, J.M.B.; Writing—original draft, F.R. and J.M.B. All authors have read and agreed to the published version of the manuscript.

References

1. FAO. "About FAO's work on plant production and protection." Rome, Italy, **2019** https://www.fao.org/plant-production-protection/about/en
2. Strange, R. N., & Scott, P. R. Plant disease: a threat to global food security. *Annu. Rev. Phytopathol.* **2005**, *43*, 83–116. doi.org/10.1146/annurev.phyto.43.113004.133839
3. Gai, Y.; Wang, H. Plant Disease: A Growing Threat to Global Food Security. *Agronomy* **2024**, *14*, 1615. https://doi.org/10.3390/agronomy14081615
4. Helps, J., Van den Bosch, F.; Sims, I.; Van den Berg, F.; Paveley, N.; Whitworth, L.; Blake, J.; Smith, J. *Septoria Tritici* Blotch in Commercial Wheat Crops in England: The Use of Fungicides for Disease Control. *Plant Pathol*. **2026**, *75*, e70158. doi.org/10.1111/ppa.70158.
5. Lavrukaitė, K.; Heick, T. M.; Ramanauskienė, J.; Armoniene, R.; Ronis, A. Fungicide sensitivity levels in the Lithuanian Zymoseptoria tritici population in 2021. *Front. Plant Sci*. **2023**, *13*, 1075038. doi: 10.3389/fpls.2022.1075038
6. McDonald, M. C., Renkin, M., Spackman, M., Orchard, B., Croll, D., Solomon, P. S., Milgate, A. Rapid Parallel Evolution of Azole Fungicide Resistance in Australian Populations of the Wheat Pathogen *Zymoseptoria tritici*. *Appl. Environ. Microbiol.* **2019**, *85*, e01908-18. doi.org/10.1128/AEM.01908-18
7. Jørgensen, L. N., Matzen, N., Heick, T. M., O'Driscoll, A., Clark, B., Waite, K., Blake, J., Glazek, M., Maumene, C., Couleaud, G., Rodemann, B., Weigand, S., Bataille, C., R, B., Hellin, P., Kildea, S., Stammler, G. Shifting sensitivity of septoria tritici blotch compromises field performance and yield of main fungicides in Europe. *Front. Plant Sci.* **2022**, *13*, 1060428. doi.org/10.3389/fpls.2022.1060428
8. Heick, T.M.; Justesen, A.F.; Jørgensen, L.N. Anti-resistance strategies for fungicides against wheat pathogen *Zymoseptoria tritici* with focus on DMI fungicides. *Crop Prot.* **2017**, *99*, 108-

117. doi.org/10.1016/j.cropro.2017.05.009

9. Corkley, I.; Fraaije, B.; Hawkins, N. Fungicide resistance management: Maximizing the effective life of plant protection products. *Plant Pathol*. **2022**, *71*, 150–169. doi.org/10.1111/ppa.13467
10. Makashvili, Z. Remarkable hive product: Propolis. Scientific data and suggestions concerning its composition, properties and possible use in therapeutics. In From the History of Propolis; APIMONDIA Standing Commission on Bee Keeping Technology and Equipment: Bucharest, Romania, **1978**; 249p.
11. Bankova, V. Recent trends and important developments in propolis research. *eCAM*. **2005**, *2*, 29–32. doi.org/10.1093/ecam/neh059
12. Sforcin, J. M. Biological properties and therapeutic applications of propolis. *Phytother. Res.* **2016**, *30*, 894–905. doi.org/10.1002/ptr.5605
13. Anjum, S.I.; Ullah, A.; Khan, K.A.; Attaullah, M.; Khan, H.; Ali, H.; Bashir, M.A.; Tahir, M.; Ansari, M.J.; Ghramh, H.A.; Adgaba, N.; Dash, C. K. Composition and functional properties of propolis (bee glue): A review. *Saudi J. Biol. Sci.* **2019**, *26*, 1695–1703. doi.org/10.1016/j.sjbs.2018.08.013
14. Rouvier, F.; Abou, L.; Wafo, E.; Andre, P.; Cheyrol, J.; Khacef, M.M.; Nappez, C.; Lepidi, H.; Brunel, J.M. Identification of 2,4-Di-tert-Butylphenol as an antimicrobial agent against *Cutibacterium acnes* bacteria from Rwandan propolis. *Antibiotics* **2024**, *13*, 1080. doi.org/10.3390/antibiotics13111080
15. Rouvier, F.; Abou, L.;Wafo, E.; Brunel, J.M. Linoleic fatty acid from Rwandan propolis: A potential antimicrobial agent against *Cutibacterium acnes*. *Curr. Issues Mol. Biol.* **2025**, *47*, 162. doi.org/10.3390/cimb47030162
16. Elbatreek, M.H.; Mahdi, I.; Ouchari, W.; Mahmoud, M.F.; Sobeh, M. Current advances on the therapeutic potential of pinocembrin: An updated review. *Biomed. Pharmacother*, **2023**, *157*, 114032. doi.org/10.1016/j.biopha.2022.114032
17. Rasul, A.; Millimouno, F.M.; Eltayb, W.A.; Ali, M.; Li, J.; Li, X. Pinocembrin: A novel natural compound with versatile pharmacological and biological activities. *Biomed. Res. Int.* **2013**, 379850. doi.org/10.1155/2013/379850
18. Badet, T.; Oggenfuss, U.; Abraham, L.; Mc Donald, B. A.; Croll, D. A 19-isolate reference-quality global pangenome for the fungal wheat pathogen *Zymoseptoria tritici*. *BMC Biol*. **2020**, *18*, 12. doi.org/10.1186/s12915-020-0744-3
19. Peng, L.; Yang, S.; Cheng, Y. J.; Chen, F.; Pan, S. ; Fan, G. Antifungal activity and action mode of pinocembrin from propolis against *Penicillium italicum* . *Food Sci.Biotechnol*. **2012**, *21*, 1533–1539. https://doi.org/10.1007/s10068-012-0204-0
20. Huang, S.; Zhang, C.P.; Wang, K.; Li, G.Q.; Hu, F.L. Recent Advances in the Chemical Composition of Propolis. *Molecules*. **2014**, *19*, 19610–19632. doi.org/10.3390/molecules191219610
21. Dezmirean, D.S., Pasca, D. ; Moise, A.R. ; Bobis, O. Plant Sources Responsible for the Chemical Composition and Main Bioactive Properties of Poplar-Type Propolis. *Plants*. **2020**, *10*, 22. doi.org/10.3390/plants10010022

Tables

Table 1 is available in the Supplementary Files section.

Figures

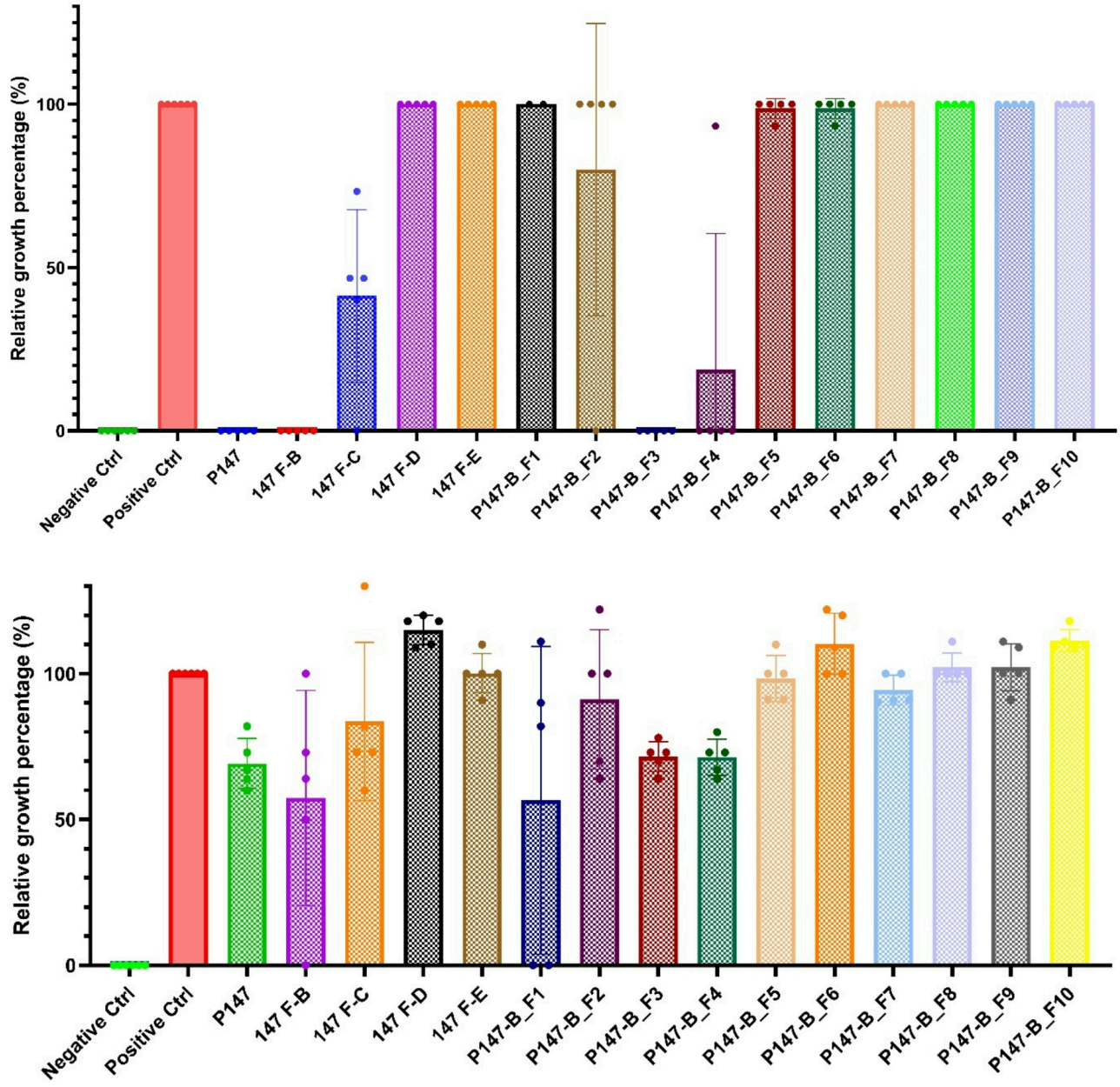


Figure 1

Antifungal activity of propolis extracts and their respective fractions. A) Growth percentages of *F. oxysporum*. B) Growth percentages of *B. cinerea*. Growth inhibition was assessed after 72 hours in triplicate at a concentration of 200µg/mL for each tested conditions

Thus, fraction **P147-B_F3** was subjected to GC–MS chemical profiling (Figure 2), which revealed the presence of pinocembrin [16-17]. This compound was unambiguously identified by comparison of its mass fragmentation pattern with the NIST library and by using the NIST 23 GC Method/Retention Index Database, which provides retention indices and gas chromatographic conditions for more than 180,000 compounds. The identification was further supported by ^{1}H and ^{13}C NMR analyses, confirming a purity greater than 95% (Figure 2). On the other hand, measurement of the optical rotation using a polarimeter gave a positive value, allowing us to conclude that the isolated product corresponds to (*S*)-pinocembrin.

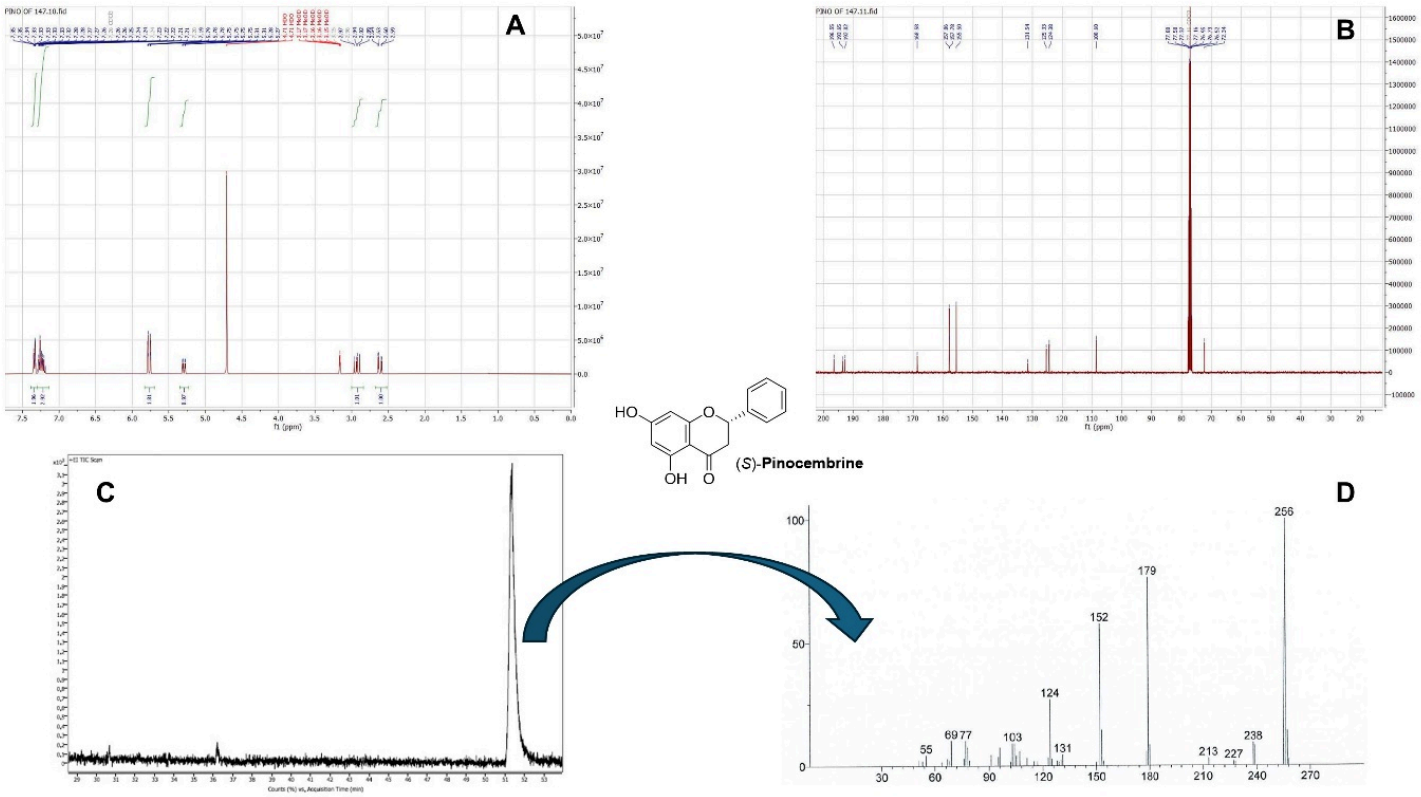


Figure 2

Determination of the identity of the molecule present in the studied fraction **P147-B_F3**. A) ^{1}H NMR analysis spectrum of fraction **P147-B_F3** B) ^{13}C NMR analysis of fraction **P147-B_F3** C) GC chromatogram of fraction **P147-B_F3** D) Mass spectrometry spectrum of fraction **P147-B_F3**

Molecule		1	2	3	4	5	
Pinocembrin	A	T-	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	*F. oxysporum*
OFAP 147	B	Te EtOH	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	
Pinocembrin	C	T-	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	*B. cinerea*
OFAP 147	D	TeOH	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	

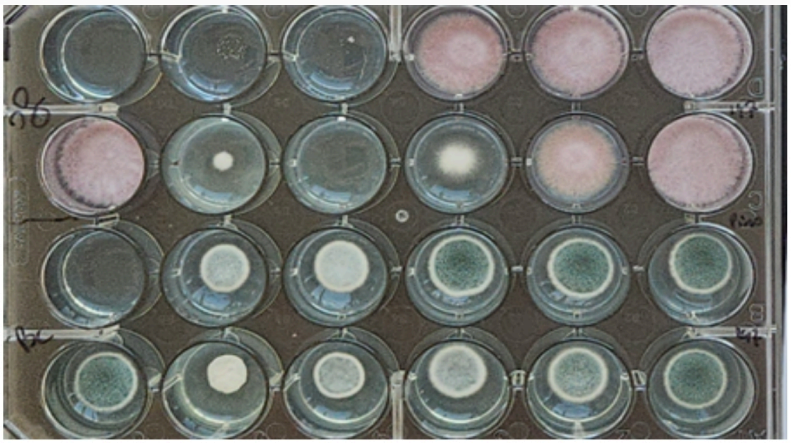


Figure 3

Antifungal activities of **OFAP147** and **(*S*)-(+)-Pinocembrin** against *F. oxysporum*and *B. cinerea* performing the tests on agar medium

Supplementary Files

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

- floatimage1.jpeg