



Hexane Extracts from *Piper aduncum* L. in Alternative *in vitro* and *in vivo* Control of Phytopathogens of Agronomical Interest

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Abstract

Even though plant extracts from *Piper aduncum* L. (Piperaceae) exhibit several biological activities, there are few reports in the literature of their application to the field of Agrochemistry. White mold, anthracnose and soft rot are diseases caused to plants by phytopathogens *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, respectively. This study had an agrochemical approach and aimed at identifying volatile constituents in hexane extracts from *P. aduncum* leaves (HE-LE) and inflorescences (HE-IN) by GC-MS and GC-FID. Their *in vitro* and *in vivo* antifungal potential was also evaluated. The former was evaluated by two methodologies: the disk diffusion method (DDM) and a modified detached leaf assay (DLA). The latter was conducted in a greenhouse with 15-day-old soybean plants. Major constituents found in HE-LE were piperitone (26.0%), β -caryophyllene (11.2%) and spathulenol (12.1%) while the ones in HE-IN were piperitone (20.0%), β -caryophyllene (24.5%) and spathulenol (14.2%). All extracts under evaluation were highly efficient at inhibiting the fungus *R. stolonifer*. In the *in vivo* assay, HE-IN was highly active since it inhibited about 97% of white mold. Thus, hexane extracts from *P. aduncum* may be considered promising to produce a natural antifungal that aims at decreasing the use of synthetic agrochemicals.

Keywords: Bioactive plant extracts. Organic Agrochemistry. Alternative control. Bioproducts.

Introduction

Plant extracts are natural products extracted from plants which exhibit a wide spectrum of biological activities. They may be used for fighting agricultural diseases because they are less toxic, safer to the environment, biodegradable and able to prevent crops from being attacked by pathogens^[1]. Thus, they are sustainable alternatives to partially or totally substitute several synthetic agrochemicals which are considered toxic^[1]. The species *Piper aduncum* L. stands out as a promising plant to be used in the field of Agrochemistry since it has remarkable chemical and biological properties that are directly related to its essential oils and botanic extracts^[2].

P. aduncum L., whose common name is spiked pepper (*pimenta-de-macaco*, in Brazilian Portuguese), belongs to the Piperaceae family which is native to tropical regions and found in several biomes, such as the Amazon, Cerrado and Pampa ones^[2]. Its extracts and essential oils have already exhibited antileishmanial, antibacterial, cytotoxic and antifungal activities^[2].

From the biological perspective, it is believed that *P. aduncum* has great potential to control agricultural fungi, such as *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, which cause severe diseases to plants, fruit and vegetables; an example is white mold, caused by *S. Sclerotiorum* to soybean plants^[3]. Anthracnose is caused by *C. gloeosporioides* while soft rot in postharvest is caused by *R. stolonifer*. These fungi lead to severe damage to several crops and losses in production, storage, transport and marketing^[3,4].

Considering that disease control in plants still depends much on the use of toxic synthetic fungicides, this study aimed at identifying volatile chemical constituents in hexane extracts from *P. aduncum* leaves (HE-LE) and inflorescences (HE-IN) by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID) (i), evaluating their *in vitro* antifungal activities against fungi *S. sclerotiorum*, *C. gloeosporioides* and *R. stolonifer* (ii) and analyzing *in vivo* antifungal activities of both hexane extracts in soybean crops to control white mold (*S. sclerotiorum*) (iii).

Material and methods

Plant material

P. aduncum L. leaves and inflorescences were collected at 9 am on February 10th, 2022, in Rio Verde, Goiás (GO), Brazil, in an environmental protection area (coordinates -17.799447S and -50.912033W) and taken to the Laboratory of Chemistry and Natural Products at the IF Goiano - *Campus* Rio Verde. The plant was identified by the biologist and botanist Luzia Francisca de Souza and a sample was kept at the Herbário Jataiense Professor Germano Guarim Neto (exsiccate no. HJ 7872). Access to the botanical material was approved by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) under the code AEACDCA.

Preparation of hexane extracts

Hexane extracts were prepared with 300 g *P. aduncum* dry leaves and 300 g *P. aduncum* dry inflorescences which were selected, washed and dried by a forced air oven for 72 h. Afterwards, they were ground by a Wiley mill to reduce granulometry and placed in flat bottom balloons. Then, 1000 mL hexane PA (C₆H₁₄)

was added. Both solvent and raw material kept in contact for four days at room temperature (26°C), protected from light and agitated daily. The resulting mixture of the extraction was separated by filtration with the use of filter paper, followed by solvent evaporation by a rotary evaporator under reduced pressure^[5]. This process led to syrup-like hexane extracts (14 g from leaves and 6 g from inflorescences).

Identification of volatile constituents of hexane extracts

Hexane extracts were dissolved in ethyl ether and analyzed by GC-FID and GC-MS with the use of Shimadzu QP5000 Plus and GCMS2010 Plus (Shimadzu Corporation, Kyoto, Japan) systems. The temperature of the column in GC-FID was programmed to rise from 60 to 240°C at 3°C/min and was held at 240°C for 5 min; the carrier gas was H₂ at flow rate of 1.0 mL/min. The equipment was set to operate in the injection mode; the injection volume was 0.1 µL (split ratio of 1:10); and injector and detector temperatures were 240 and 280°C, respectively. Relative concentrations of components were obtained by normalizing peak areas (%). Relative areas consisted of the average of triplicate GC-FID analyses. GC-MS conditions and the identification have been previously reported^[6]. Identification of volatile components of hexane extracts (**TABLE 1**) were based on their retention indices on an Rtx-5MS (30 m X 0.25 mm; 0.250 µm) capillary column under the same operating conditions used for GC relative to a homologous series of *n*-alkanes (C₈-C₂₀). Structures were computer-matched with Wiley 7, NIST 08 and FFNSC 1.2 and their fragmentation patterns were compared with literature data^[7].

Fungal strains

Both phytopathogenic fungi *S. sclerotiorum* and *R. stolonifer* were provided by the Laboratory of Phytopathology at the IF Goiano - *Campus* Rio Verde. The fungus *C. gloeosporioides* was isolated from contaminated papaya at the Laboratory of Phytopathology at the IF Goiano - *Campus* Rio Verde.

In vitro antifungal activities of HE-LE and HE-IN by the disk diffusion method

A standard solution was prepared with 4 g plant extract and 10 mL ethyl acetate to reach concentration 1 (C1 - 0.4 g/mL or 400 ppm). Eight mL of the standard solution was poured into a test tube (C1) to reach concentration 2 (C2 - 0.2 g/mL or 200ppm). Four mL ethyl acetate and 4 mL of concentration 1 (C1) were poured into another test tube and agitated to dilute completely and reach concentration 3 (C3 - 0.1 g/mL or 100 ppm). Concentration 4 (C4 - 50 ppm) resulted from the addition of 4 mL ethyl acetate and 4 mL of concentration 3, which was mixed in another test tube. Concentration 5 (C5 - 0.025 g/mL or 25 ppm) resulted from dilution of 4 mL ethyl acetate and 4 mL of concentration 4. Controls of the experiment were ethyl acetate (B - 0 ppm) and the one with no plant extract at all (T). Biological assays were carried out by a flow chamber at the Laboratory of Phytopathology at the IF Goiano - *Campus* Rio Verde. Petri dishes were sterilized by an autoclave and filled with potato dextrose agar (PDA) medium. Then, 0.50 µL of diluted solutions was uniformly spread on them with a Drigalski spatula. After that, 5-mm-diameter disks of PDA medium with mycelia of every fungus were placed in the center of all dishes. They were then stored in Biological Oxygen Demand (BOD) at 21°C up to fungus growth, following the methodology described by Rezende *et al.*^[8]. Growth was finally measured after total fungal growth on control dishes (seven days).

Percentage of inhibition of mycelial growth (IMG) was calculated by the following formula^[9]:

$$IMG (\%) = \frac{(\text{control growth} - \text{treatment growth})}{\text{control growth}} \times 100$$

***In vitro* antifungal activities of HE-LE and HE-IN by a modified detached leaf assay**

The detached leaf assay followed the methodology proposed by Gabardo *et al.*^[9] with some adaptations. Soybean leaves were detached and placed on Petri dishes with filter paper and water to keep them humid. Afterwards, leaves were inoculated with 5-mm-diameter disks of *S. sclerotiorum* culture grown in PDA medium for four days and were incubated in a germination chamber at 22 ± 3°C for 24 h under a 12-h photoperiod in a BOD incubator. After the incubation period, soybean leaves were sprinkled with their respective experimental treatments. Solutions of hexane extracts were prepared with distilled water, ethyl acetate and tween 80 to reduce toxicity in soybean leaves. Solutions that had the most active concentrations were HE-LE (C1 - 0.4 g/mL and C2 - 0.2 g/mL) and HE-IN (C1 - 0.4 g/mL and C2 - 0.2 g/mL). Besides, both treatments were used: control (B - 0 g/mL) and control with no treatment (T). Right after hexane extracts were sprinkled, Petri dishes were incubated again at 22 ± 3°C under a 12-h photoperiod in BOD. The first evaluation started after 12 h of incubation and was carried out until total growth of the control with no treatment (T). The leaf lesion was measured by a digital pachymeter.

Outlining and statistics of *in vitro* assays

The experiment had a completely randomized design in a factorial scheme which consisted of two types of extracts from *P. aduncum* (leaves and inflorescences), three species of fungi (*S. sclerotiorum*, *C. gloeosporioides* and *R. stolonifer*) and seven treatments composed of different concentrations of hexane extracts. Concentrations resulted from dilution of plant extract in ethyl acetate PA in a serial dilution scheme at the following concentrations: 0.4; 0.2; 0.1; 0.05; 0.025 g/mL, the solvent ethyl acetate (white) and the control with no extract with five replicates per treatment, thus, totaling 210 experimental units (2x3x7x5=210). Data on percentages of IMG were subject to the normality test which showed the normal distribution of parameters under evaluation. Regarding descriptive statistics, data were subject to the analysis of variance (ANOVA), the Tukey's test (p<0.05) and the R Core Team 8.0 software program. Results were expressed as tables.

***In vivo* antifungal assay in soybean plants**

Glycine max plants, super-early cycle, were grown in 2-kg plastic bags filled with substrate – sand and NPK (4-30-16) – and were kept in a greenhouse at room temperature and manual irrigation for 15 days under a 12-h photoperiod. The methodology proposed by Garcia *et al.*^[10] was applied. Fifteen-day-old soybean plants were taken to the Laboratory of Chemistry and Natural Products at the IF Goiano - *Campus* Rio Verde to have their leaves inoculated with the fungus. Inoculation took place when 7-mm-diameter disks with the fungus grown in PDA medium for four days were inserted into the adaxial sides of leaves. After inoculation, plants were kept at 22°C ± 3°C at 90% humidity for 24 h under a 12-h photoperiod to enable early fungal growth. After 24 h, soybean plants were treated with the solutions of the most active extracts at the same concentrations employed in the detached leaf assay. The most active concentrations were HE-LE (C1 - 0.4 g/mL and C2 - 0.2 g/mL) and HE-IN (C1 - 0.4 g/mL and C2 - 0.2 g/mL). In addition, both the control (B - 0 g/mL) and the control with no treatment (T) were evaluated. Right after the application of sprinkle treatments with the use of a manual sprinkler, leaves were kept at 22°C ± 3°C at 90% humidity under a 12-h photoperiod. Areas of leaf lesions were measured by a digital pachymeter 24 h after application of treatments up to total growth of *S. sclerotiorum* on leaves of plants in the negative control.

Outlining and statistical analysis of *in vivo* assays

In vivo experiments had a completely randomized design in a factorial scheme that consisted of six treatments: control, control with no treatment and the four most active concentrations of HE-LE and HE-IN, with five replicates per treatment, totaling 60 experimental units (2x6x5=60). Data on percentages of IMG were subject to the normality test (Shapiro-Wilk)^[11].

Results and Discussion

Volatile constituents of HE-LE and HE-IN were identified by GC-FID and GC-MS. Total percentages of constituents identified in HE-LE and HE-IN were 98.2% and 97.1%, respectively. Major constituents found in HE-LE were piperitone (26.0%), β -caryophyllene (11.2%) and spathulenol (12.1%) (**TABLE 1**) while the ones identified in HE-IN were piperitone (20.0%), β -caryophyllene (24.5%) and spathulenol (14.2%) (**TABLE 1**).

TABLE 1: Volatile constituents identified in HE-LE and HE-IN from *P. aduncum* (Piperaceae).

Compound	RT (min)	RI _{exp}	RI _{lit}	% RA	
				HE-LE	HE-IN
Terpinen-4-ol	20.57	1180	1179	10.0	10.5
Piperitone	24.31	1252	1254	26.0	20.0
α -cubebene	33.57	1350	1351	0.9	0.2
β -elemene	34.59	1373	1375	1.9	2.3
Isodene	34.76	1376	1377	0.5	—
β -cubebene	35.33	1389	1390	6.5	—
β -caryophyllene	36.43	1416	1418	11.2	24.5
γ -elemene	36.78	1425	1423	5.0	—
Aromadendrene	37.10	1438	1439	2.1	1.0
α -selinene	37.71	1449	1451	—	1.6
α -humulene	37.94	1455	1455	0.1	—
Alloaromadendrene	38.12	1459	1460	—	2.0
β -Cadinene	38.58	1471	1472	0.1	5.2
γ -muurolene	38.76	1475	1476	1.3	1.0
β -selinene	38.88	1483	1485	6.8	—
Valencene	39.38	1491	1491	4.5	3.4
Bicyclogermacrene	39.56	1493	1493	2.9	3.5
α -Cadinene	41.15	1537	1538	1.0	—
Germacrene B	41.95	1559	1560	3.1	0.7

Compound	RT (min)	RI _{exp}	RI _{lit}	% RA	
				HE-LE	HE-IN
Spathulenol	42.48	1574	1575	12.1	14.2
Isoaromadendrene oxide	42.71	1578	1579	—	2.0
Caryophyllene oxide	42.89	1583	1581	0.8	0.5
Viridiflorol	43.16	1589	1590	0.6	—
Humulene epoxide	43.93	1610	1609	—	0.9
Isospathulenol	44.23	1630	1631	0.8	—
α-muurolol	45.19	1645	1645	—	1.1
Aromadendrene oxide	45.91	1667	1668	—	1.8
Alloaromadendrene oxide	47.03	1696	1697	—	0.7
Total				98.2	97.1

RT = Retention time; **RI_{exp}** = Retention index relative to *n*-alkanes (C₈–C₂₀) on the Rtx-5MS column; **RI_{lit}** = Kovats retention index (values from the literature^[7]). **%RA** = Relative abundance. -: not detected. Bold numbers mean that volatile constituents were considered major constituents of hexane extracts.

Results of this study disagree with the ones found by Lucena *et al.*^[12], whose GC-MS analysis showed that hexane extract from *P. aduncum* leaves was mainly composed of apiole (90.7%). It should be highlighted that apiole, a phenylpropanoid, was identified neither in HE-LE nor in HE-IN from this species found in the Cerrado Goiano Biome. A study conducted by Santos and collaborators^[13] reports that major constituents of hexane extract from *P. aduncum* were caryophyllene, α-calacorene, γ-elemene, *cis*-γ-elemene, germacrene D, linalool, linalool oxide, nerolidol, β-elemene, δ-cadinene, α-caryophyllene and falcarinol. The fact that some chemical constituents are found in some extracts from *P. aduncum* but not in others may be explained by activities of biotic and abiotic factors, such as soil, seasonality, harvest period, material preparation and part of plants under study^[14].

Different concentrations of HE-LE against *S. sclerotiorum*, *C. gloeosporioides* and *R. stolonifer* exhibited low coefficients of variation in statistical analyses. Treatments with the highest concentrations – 0.4 and 0.2 g/mL in treatments C1 and C2, respectively – did not differ statistically from each other and exhibited the highest mean values of inhibition against the three fungi under evaluation. *R. stolonifer* exhibited the highest inhibition since mean values were about 80% at the highest doses (C1 and C2). *S. sclerotiorum* exhibited lower values of inhibition in all treatments; they did not reach 50% of mycelial inhibition. *C. gloeosporioides* had its best result at concentration C1 since inhibition reached 80.73%. Finally, both the control (B) and the control with no treatment (T) were statistically equal since there was no fungal inhibition (**TABLE 2**).

TABLE 2: *In vitro* antifungal activity of HE-LE by the disk diffusion method.

Fungi	% inhibition						
	C1	C2	C3	C4	C5	B	T
SSC	48.02 b	43.53 c	17.88 c	8.18 a	0.0 b	0.0 a	0.0 a
RST	94.42 a	93.78 a	73.87 a	74.03 a	61.64 a	0.0 a	0.0 a
CGL	80.73 c	67.78 b	63.78 b	17.28 b	0.0 b	0.0 a	0.0 a

*Means followed by the same letter in a column do not differ statistically from each other by the Tukey's test at 5% probability ($p=0.005$). SSC: *S. sclerotiorum*. RST: *R. stolonifer*. CGL: *C. gloeosporioides*.

In assays with HE-IN, concentrations 0.4 and 0.2 g/mL, treatments C1 and C2, did not differ statistically from each other and exhibited high mean values of fungal inhibition. HE-IN was able to inhibit 100% of *S. sclerotiorum* growth (TABLE 3). Against *C. gloeosporioides*, the best result of inhibition – 80.73% – was reached at C1. This percentage was also reached by HE-LE. It should be emphasized that HE-IN was more efficient against *R. stolonifer* since inhibition was about 60% even at the lowest concentration, i. e., 0.05 g/mL (C4) (TABLE 3). It should also be mentioned that, in both control (B) and control with no treatment (T), all fungi exhibited total development, a fact that reassures that ethyl acetate is not fungitoxic and did not affect results of *in vitro* assays.

TABLE 3: *In vitro* antifungal activity of HE-IN by the disk diffusion method.

Fungi	% inhibition						
	C1	C2	C3	C4	C5	B	T
SSC	100 a	100 a	53.65 a	46.62 b	36.62 a	0.0 a	0.0 a
RST	89.60 b	83.73 b	73.04 a	69.40 a	0.0 b	0.0 a	0.0 a
CGL	80.73 c	67.56 c	63.78 b	17.28 c	0.0 b	0.0 a	0.0 a

*Means followed by the same letter in a column do not differ statistically from each other by the Tukey's test at 5% probability ($p=0.005$). SSC: *S. sclerotiorum*. RST: *R. stolonifer*. CGL: *C. gloeosporioides*.

Results of this study of HE-LE and HE-IN agree with and complement the study carried out by Valadares and collaborators^[4], who showed that essential oils from *P. aduncum* leaves and inflorescences were highly active against *S. sclerotiorum*. The fact that hexane extracts and essential oils are liposoluble enables their permeability through cell membranes, leading to cell death due to loss of ions and cell material and, consequently, preventing fungal development from taking place^[15]. Another reason that may explain the fact that HE-IN exhibits the best antifungal activity is its high concentration of the chemical constituent β -caryophyllene (24.5%), a powerful *in vitro* antifungal against phytopathogens^[16]. β -caryophyllene is a hydrophobic molecule, which presents low molecular weight; these properties facilitate the permeability in the cellular membrane of the fungi, being able to be involved in the mechanism of action of inhibition of microbial growth^[16]. Besides, HE-IN exhibits the sesquiterpene spathulenol in higher concentration (14.2%). Spathulenol may also reinforce that activity exhibited by HE-IN is better than the one shown by HE-LE since the chemical constituent has promising antimicrobial potential^[17]. Furthermore, piperitone was able to favor mycelial inhibition of different fungi according to the literature^[18].

In the detached leaf assay applied to soybean plants, with the use of HE-LE and HE-IN, treatments with the highest doses of extracts (C1 and C2) significantly inhibited fungal growth and leaf necrosis. This analysis

was carried out five days after inoculation of disks with the chosen fungus, i. e., *S. sclerotiorum* (**FIGURE 1**). HE-LE exhibited higher viscosity and lower effectiveness and versatility than HE-IN throughout application. Treatments with no extracts – control (B) and control with no treatment (T) – exhibited high level of damage to leaf tissues as the result of fast fungal contamination; fungal mycelia may be easily seen in **FIGURE 1**. Results of the detached leaf assay are shown in **TABLE 4**.

FIGURE 1: Soybean leaves in the detached leaf assay after application of the most active extracts. Photographs were taken five days after they were inoculated with *S. sclerotiorum*. 1. leaf with no treatment totally attached by the fungus; 2. leaf sprinkled with HE-IN at concentration C2; and 3. leaf sprinkled with HE-IN at concentration C1.



TABLE 4: *In vitro* evaluation of antifungal activities of HE-LE and HE-IN against *S. sclerotiorum* by the detached leaf assay.

% inhibition	
<i>S. sclerotiorum</i>	
HE-LE [C1]	43.86 c
HE-LE [C2]	51.01 b
HE-IN [C1]	99.07 a
HE-IN [C2]	98.96 a
B	0.0 d
T	0.0 d

* Means followed by the same letter in a column do not differ statistically from each other by the Tukey's test at 5% probability ($p=0.005$). [C1] and [C2] are the highest concentrations used in the assays.

HE-LE exhibited low percentages of inhibition of mycelial growth, i. e., 43% (C1) and 51% (C2), while HE-IN had relevant results at the same concentrations – around 98% - and showed its high control over *S. sclerotiorum*. This data reveals the promising potential of HE-IN as a fungicide. It should be noted that detached leaf assays are very promising to determine efficiency of extracts to control diseases since conditions in which pathogens colonize plants, such as temperature and humidity, are excellent and favor maximum expression of diseases in plant tissues. This fact was shown by Fonseca *et al.*^[19] who stated that

diseases caused by *Rhizoctonia solani* and *Sclerotium rolfsii* were satisfactorily controlled in detached leaves since extracts under evaluation were as active as the commercial fungicide used by their study.

In vivo assays in soybean showed little difference in percentages of fungal inhibition by comparison with the detached leaf assay. Application of HE-IN to soybean plants led to effective inhibition of *S. sclerotiorum* growth (around 97%; **TABLE 5**). Besides, neither necrosis nor sick areas were formed (**FIGURE 2**) shows that the control plant was completely consumed by white mold whereas the plant treated with HE-IN at concentration C1 kept healthy major structures. HE-LE exhibited poor inhibitory activity, whose values were 40.42% (C1) and 42.33% (C2) (Table 5). Once again, control (B) showed that ethyl acetate, which was used for diluting the extracts, did not interfere with fungal growth.

TABLE 5: *In vivo* inhibitory activity exhibited by HE-LE and HE-IN against *S. sclerotiorum* in soybean plants.

% inhibition	
<i>S. sclerotiorum</i>	
HE-LE [C1]	40.42 b
HE-LE [C2]	42.33 b
HE-IN [C1]	98.84 a
HE-IN [C2]	97.54 a
B	0.0 c
T	0.0 c

Means followed by the same letter in a column do not differ statistically from each other by the Tukey's test at 5% probability (p=0.005). [C1] and [C2] are the highest concentrations used in the assays.

FIGURE 2: Soybean plants attacked by *S. sclerotiorum* inoculum in treatments: control with no treatment (left) and HE-IN at concentration C1 (right).



In vitro and *in vivo* assays have proven that HE-IN has promising antifungicidal activity against the fungus that causes white mold to soybean. It is implied that viscosity and syrup-like consistency of HE-LE may have interfered negatively with results of the assays. Besides, variation in fungal inhibition resulting from concentrations of hexane extracts showed the relation of dependence between concentrations of natural products and inhibition of agricultural fungi. Concerning plant extracts, the literature reports that the higher

the concentration of bioactive secondary metabolites and the synergy among these compounds, the more potentialized the fungitoxic property^[20].

On the other hand, it should be highlighted that *in vivo* assays with plants are subject to biological responses given by the plants themselves against phytopathogens. Plants exhibit mechanisms of defense when they are attacked by pathogens and start to respond immediately after the attack by producing specific compounds as defense and resistance, thus, leading to abortion of injured or contaminated structures^[21]. However, studies of plant extracts that aim at controlling fungi that attack plants are important in the field of Agrochemistry since they enable family and organic farmers to use alternative, sustainable and less toxic solutions^[20].

Conclusion

The major constituents found in HE-LE were piperitone, β -caryophyllene and spathulenol while the ones in HE-IN were the same. In the conditions of the biological experiments, the conclusion is that HE-IN exhibits the best inhibitory activity over mycelial growth of fungi under evaluation while HE-LE showed low activity in *in vitro* and *in vivo* assays. HE-IN was highly active in all assays, but HE-LE was not. In sum, this study contributed positively to further studies of the species *P. aduncum* so as to promote the development of natural agrochemicals based on bioactive compounds from plants.

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Conflicts of Interest

The authors declare no conflict of interest.

Collaborators

Funding acquisition and supervision: CCFA; MLDM.

Conceptualization, investigation, methodology: VPM; MNX; AEMC; JGB; MSA.

Writing, original draft and Writing, review & editing: MLDM.

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