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Evaluation of Pesticidal Activities of Lignans Isolated from *Piper cubeba* Fruits

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Abstract

The identification of natural, plant-derived compounds with pesticidal properties is crucial for developing environmentally sustainable alternatives to synthetic pesticides. In this study, four major lignans—dihydroclusin, cubebin, clusin, and yatein—were isolated from the crude extract of *Piper cubeba* fruit. Phytotoxicity assays revealed herbicidal activity against *Agrostis stolonifera*, with dihydroclusin and clusin exhibiting the highest efficacy, inhibiting seed germination by 50% and showing IC_{50} values of 2.9 μM and 45 μM, respectively, against *Lemna paucicostata*. Additionally, all compounds, except dihydroclusin, demonstrated fungicidal activity against the strawberry anthracnose pathogen *Colletotrichum fragariae*. Moreover, only dihydroclusin exhibited larvicidal activity against *Aedes aegypti*, causing 96% mortality of mosquito larvae at the 100-ppm concentration tested. These findings highlight the broad-spectrum bioactivity of *Piper cubeba* lignans, suggesting their potential as alternative agents of synthetic pesticides for managing agricultural pests.

Keywords

Piper cubeba, Lignans, Herbicidal, Fungicidal, Larvicidal

1. Introduction

Pesticides are crucial tools in modern agriculture, acting as essential components for pest, disease, and weed management. Their application aims to protect crops and increase yields, ensuring food security and the quality of agricultural products [1]. The use of pesticides significantly contributes to global agricultural production, protecting crops from substantial losses. Despite their importance, pesticide use presents considerable challenges. Excessive, repeated and improper use of

these products can generate various negative effects on the environment, and human health [2]. To mitigate the negative impacts of pesticides, it is necessary to seek alternative and sustainable solutions for pest and disease management in agriculture.

Natural products emerge as a promising alternative for pest and disease management in agriculture. Compared to chemical pesticides, their inherent environmental and human health benefits make them a crucial component of sustainable agricultural practices [3]. As global concerns over environmental pollution escalate, the adopting of natural products represents a paradigm shift toward more eco-friendly and sustainable agricultural practices, ensuring the preservation of both natural resources and agricultural productivity [4]. The development of compounds exhibiting herbicidal, fungicidal, and larvicidal activities is of a great importance for sustainable agriculture and public health. Herbicidal compounds are crucial for effective weed management, reducing competition for nutrients, light, and space, thereby enhancing crop yields and quality [5]. Fungicidal compounds play a vital role in controlling phytopathogenic fungi, preventing significant crop losses due to diseases [6]. Additionally, larvicidal compounds targeting *Aedes aegypti* are essential for controlling the spread of vector-borne diseases such as dengue, zika, and chikungunya. Effective larvicides reduce the population of mosquito larvae, thereby decreasing the incidence of these diseases [7].

Piper cubeba L.F., commonly known as cubeb pepper or tailed pepper, is a plant species belonging to the Piperaceae family, native to Southeast Asia. *P. cubeba* has been historically utilized as a culinary spice and traditional medicinal ingredient [8]. Its dried fruits, resembling small spherical tails, contain bioactive compounds such as cubebol and cubebin belonging to the sesquiterpene alcohol and lignan classes, respectively. Cubebol is used as a refreshing agent and is also used in the flavor industry and has been studied for its potential insecticidal and larvicidal activities [9] [10]. Cubebin, on the other hand, exhibits significant medicinal properties, including anti-inflammatory, analgesic, and anticancer activities [11].

In traditional herbal medicine, *P. cubeba* is esteemed for its diuretic, antimicrobial, and digestive-stimulating properties, with its anti-inflammatory activity being particularly notable. Several *in vitro* and *in vivo* studies support these biological applications, highlighting the plant's therapeutic potential [12]. Additionally, *P. cubeba* is found to be used as a flavoring agent in food and beverage industries, contributing a distinctively pungent and spicy flavor profile to culinary creations. *P. cubeba* remains a valuable botanical resource with diverse cultural, culinary, and medicinal significance [12].

P. cubeba is also a source of lignans, a class of bioactive compounds with potential as natural pesticides [13]. Studies have indicated that lignans derived from *P. cubeba* exhibit significant insecticidal and fungicidal activities against various agricultural pests and pathogens. The extract of *P. cubeba* has demonstrated antifungal activity against spoilage fungi isolated from tomatoes, grapes, and lemons. Additionally, the extract and isolated compounds have shown promising

antibacterial and sporicidal activities against *Bacillus* species [14] [15]. Furthermore, *P. cubeba* fruits and their lignans have exhibited potential larvicidal activity against *A. aegypti* larvae, causing structural damage to the larvae's tegument [16]. These findings suggest that *P. cubeba* and its lignans are promising candidates for pest management strategies. This study aimed to investigate the herbicidal, fungicidal and larvicidal activities of lignans isolated from *P. cubeba*.

2. Materials and Methods

2.1. Extraction and Isolation of Major Lignans from *P. cubeba*

P. cubeba seeds bought from Floral Seeds (New Delhi, India) were processed following the methodology described by Arruda *et al.* [17]. The seeds were dried and powdered, then 10 g were subjected to ultrasound-assisted extraction with 500 mL of 84% ethanol for 40 minutes at room temperature using an ultrasonicator. The extraction was subsequently filtered and concentrated under a vacuum using a rotary evaporator, furnishing 0.9 g of crude extract, a yield of 9%.

For the fractionation process, 250 mg of the crude extract was submitted to the automatized flash chromatography process, using a Biotage® flash chromatography system equipped with a quaternary pump and a diode array detector set to 254 nm and 280 nm wavelengths using Biotage® Ultra 30 µm cartridge (25 g) with hexane and ethyl acetate as mobile phase. The obtained fractions were analyzed using thin-layer chromatography plates (250 µm silica gel plates, Analtech, Newark, DE, USA) with hexane: ethyl acetate (7:3) and derivatization with p-anisaldehyde/sulfuric acid.

Further purification of the fractions was performed using a Prominence preparative HPLC (Shimadzu, Kyoto, Japan) interfaced with an SPD-20A ultraviolet (UV) detector and an FCR-10A automatic sample collector. The column was a 250 × 10 mm inner diameter, 4 µm, semi-preparative C_{18} (Phenomenex, Torrance, CA, U.S.A.). Each fraction was solubilized in methanol at a concentration of 50 µg/mL and then 100 µL of this solution was injected in each run. The mobile phase consisted of acidified water (0.3% formic acid) and acetonitrile with a flow of 4 mL/min. The gradient method was used as described by Arruda *et al.* [17].

The isolated compounds were identified by nuclear magnetic resonance (NMR). The 1D and 2D NMR spectra of the isolated compounds were recorded using a Bruker Avance III-400 MHz spectrometer, with $CDCl_3$ as solvent. Optical rotation $[\alpha]_D$ of the compounds was recorded in autopol IV (Rudolph research analytical, Nova Jersey, USA) digital polarimeter with sample concentration at 1 mg/mL in chloroform at 25˚C. Direct analysis of purified compounds in real time-high-resolution mass spectrometry (DART-HRMS) was conducted using an AccuTOF-DART mass spectrometer (JEOL USA, Inc., Peabody, MA, USA).

Compound (1) (Dihydroclusin) as white powder. $[\alpha]_D^{25}$: +3.98 (c 1.00, $CHCl_3$). High-resolution DART positive m/z 405.3342 $[M + H]^+$, calculated for $C_{22}H_{29}O_7$ 405.1913. ^1H NMR (400 MHz, $CDCl_3$) δ 6.72 (*d*, *J* = 7.8 Hz, 1 H), 6.64 (*d*, *J* = 1.4 Hz, 1H), 6.60 (*dd*, *J* = 7.8, 1.4 Hz, 1 H), 6.35 (*d*, *J* = 2.4 Hz, 2 H), 5.90 (*s*, 2H), 3.84 - 3.55 (*dd*, *J* = 11.3, 3 - 9 Hz, 4 H), 3.82 (*s*, 9 H), 2.75 - 2.65 (*m*, 4 H), 1.90 (*m*, 2

H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 147.7, 145.8, 136.6, 136.3, 134.3, 121.9, 109.3, 108.1, 106.1, 100.8, 60.9, 60.8, 56.2, 56.1, 44.1, 43.8, 36.66, 35.9 [18].

Compound (2) (Cubebin) as white powder. $[\alpha]_D^{25}$: −12.23 (c 1.00, $CHCl_3$). High-resolution DART positive m/z 357.1313 $[M + H]^+$, calculated for $C_{20}H_{21}O_6$ 357.3820. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 – 6.74 (*m*, 6H), 5.90 (*s*, 4H), 5.23 (*s*, 1 H), 4.11 (*t*, *J* = 7.76 Hz, ½ H), 4.01 (*t*, *J* = 7.76 Hz, ½ H), 3.87 (*t*, *J* = 7.86 Hz, ½ H), 3.58 (*t*, *J* = 7.86 Hz, ½ H), 2.44 – 2.76 (*m*, 4 H), 1.96 – 2.16 (*m*, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.5, 147.5, 145.9, 145.7, 134.1, 133.3, 121.6, 121.4, 109.3, 108.9, 108.1, 103.3, 100.8, 72.6, 53.1, 45.9, 39.2, 33.6 [19].

Compound (3) (Clusin) as a yellow resin. $[\alpha]_D^{25}$: −39.78 (c 1.00, $CHCl_3$). High-resolution DART positive m/z 403.1764 $[M + H]^+$, calculated for $C_{22}H_{27}O_7$ 403.4510. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.73 – 6.32 (*m*, 6 H), 5.90 (*m*, 2 H), 5.23 (*d*, *J* = 4.8 Hz, 1 H), 4.12 (*t*, *J* = 8.1 Hz, ½ H), 4.03 (*t*, *J* = 8.3 Hz, 1 H), 3.59 (*t*, *J* = 8.0 Hz, ½ H), 3.82 (*s*, 9 H), 3.78 (*dd*, *J* = 11.7, 1.7 Hz, 1 H), 3.51 (*dd*, *J* = 11.4, 4.3 Hz, 1 H), 2.77 – 2.64 (*m*, 2 H), 2.47 – 2.18 (*m*, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 147.8, 146.0, 136.6, 135.3, 134.1, 121.9, 109.3, 108.9, 108.1, 106.0, 100.9, 72.6, 60.9, 56.2, 53.0, 46.1, 39.1, 35.9 [20].

Compound (4) (Yatein) as a colorless oil. $[\alpha]_D^{25}$: −6.73 (c 1.00, $CHCl_3$). High-resolution DART positive m/z 401.1601 $[M + H]^+$, calculated for $C_{22}H_{25}O_7$ 401.1600. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (*d*, *J* = 8.0 Hz, 1 H), 6.59 (*m*, 1 H), 6.47 (*m*, H-6, 2 H), 6.35 (*s*, 1 H), 5.93 (*m*, 2 H), 4.18 – 3.88 (*m*, 2 H), 3.83 (*m*, 9 H), 2.96 (*ddd*, *J* = 16.0, 8.0, 3.1 Hz, ½ H), 2.91 (*dd*, *J* = 8.0, 6.0 Hz, 1 H), 2.85 (*ddd*, *J* = 10.6, 8.0, 3.0 Hz, ½ H), 2.56 (*m*, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5, 153.3, 148.0, 146.5, 137.15, 133.3, 131.6, 121.5, 108.8, 108.2, 106.4, 101.1, 71.2, 60.8, 56.2, 46.5, 41.1, 38.4, 35.3 [18].

2.2. Herbicidal Evaluation of Isolated Compounds on *Lactuca sativa* L. and *Agrostis stolonifera* L.

Isolated compounds were assessed for their phytotoxic effects on lettuce (*Lactuca sativa*) and bentgrass (*Agrostis stolonifera* Leers) seeds, following the protocol described by Dayan *et al.* [17]. Seeds of *Lactuca sativa* (Iceberg A Crisphead from Burpee Seeds, Pensilvania, USA) and *Agrostis stolonifera* (Penncross variety, belonging to the Creeping bentgrass species, sourced from Turf-Seed, Inc. in Hubbard, Oregon, USA) were first surface sterilized by immersing in a 0.5% - 1% sodium hypochlorite for approximately 10 minutes. The seeds were then thoroughly rinsed with sterile deionized water and air-dried in a sterile environment.

In a 24-well plate (Corning Inc., Corning, NY), each well contained *A. stolonifera* seeds (10 mg) or *L. sativa* (5 seeds) placed on a filter paper (Whatman no. 1). The test compounds and fractions were dissolved in a mixture of acetone and Deioninezed (DI) water, ensuring a final acetone concentration of 10%. Subsequently, 200 µL of the test solution was added to each well containing seeds, while the control wells received only 200 µL of acetone and DI water. A 1 mM atrazine (ChemServices) solution served as the positive control. The plate was covered and

sealed using Parafilm and placed in a Percival Scientific CU-36L5 incubator, with continuous light conditions at 26˚C and an average photosynthetically active radiation (PAR) photon flux of 120 μmol·s^{-1}·m^{-2}. The phytotoxic activity was qualitatively assessed by visual comparison of seed germination in each well after 7 days for *L. sativa* and 10 days for *Agrostis stolonifera*, using a rating scale ranging from 0 to 5. A rating of 0 indicated no effect (all seeds germinated), while a rating of 5 indicated no seed germination [21]. Each experiment was conducted in triplicate.

2.3. Herbicidal Assay with *Lemna paucicostata*

Lemna paucicostata cultures were cultivated from a single colony, comprising a mother and two daughter fronds, in a beaker filled with Hoagland's No. 2 Basal Salt Mixture (Sigma H2395) at a concentration of 1.6 g/L, supplemented with iron (1 mL of 1000 × FeEDTA solution per 1 L of Hoagland media). The pH of the medium was adjusted to 5.5 using 1 N NaOH and then filter-sterilized through a 0.2 μm filter. These *L. paucicostata* cultures were grown in approximately 100 mL of sterile jars with vented lids in a Percival Scientific CU-36L5 incubator, maintaining continuous light conditions at 26˚C and an average PAR photon flux of 120 μmol·s^{-1}·m^{-2}. The doubling time for the plants was approximately 24 h to 36 h. Nonpyrogenic polystyrene sterile six-well plates (CoStar 3506, Corning Incorporated) were used for assays. Each well contained 4950 μL of Hoagland's media and 50 μL of water, solvent, or the compound dissolved in the appropriate solvent, resulting in a final solvent concentration of approximately 1% by volume. Atrazine was used as the positive control. LemnaTec image analysis software utilized a graphical template of the six-well plates. Two three-frond plants of the same age (four to five days old) and approximate size were inoculated into each well. As mentioned earlier, all six-well plates were placed in the Percival incubator, maintaining conditions at 26˚C and 120 μmol·s^{-1}·m^{-2} average PAR photon flux. Plant measurements were recorded on day 0, day 7, and various days in between using the LemnaTec image analysis methodology [21]. The IC_{50} value was generated based the dose response curve of the calculated growth rate with the support of R software and drc package. The dose-response curves were plotted in the GraphPad software 10.2.2.

2.4. Antifungal Bioautography Assay

The assessment of antifungal activity against fungal plant pathogens followed a published TLC bioautography method [22]. We selected a fungal crop pathogen *Colletotrichum fragariae* (isolate CF63) that infects strawberries as well as other vegetables and fruits. Pure compounds were dissolved in methanol and then 10 μL was applied at concentrations of 10, 20, 50, and 100 μM into silica gel TLC plates (250 μm, silica gel GF Uniplate; Analtech, Inc.). After solvent evaporation, the plates were then sprayed with spore suspensions of *C. fragariae*, adjusted to a final concentration of 3.0 × 10^5 conidia/mL in potato dextrose broth (PDB, Difco)

and 0.1% Tween-80. The TLC plates were sprayed until damp with the prepared conidial suspension. The inoculated TLC plates, placed in a moisture chamber (30 × 13 × 7.5 cm) boxes to maintain 100% relative humidity, were incubated in a growth chamber for 4 days kept at 27˚C ± 1˚C, with a 12-h photoperiod under photon flux conditions of 60 ± 5 μmol $s \cdot m^{-2} \cdot s^{-1}$. To determine the sensitivity of each tested compound against fungal species, inhibitory zone areas were compared. Bioautography experiments were conducted in triplicate, including both dose-response and non-dose-response assessments. A technical grade fungicide standard, captan (98%; Chem Service, Inc.), was the positive control. Clear or diffuse zones of fungal growth inhibition on the TLC plate indicated the fungicidal effectiveness of the compounds and were measured using a digital caliper (Neiko®). The means and standard deviations of the triplicates were recorded and presented in Table 2.

2.5. Larval Bioassay of Compounds

All compounds were solubilized in DMSO with gentle heating and agitation and then used to create 10000 and 2000 ppm stocks. The larval bioassay was conducted using the pesticide susceptible ORL1952 strain of first instar *Aedes aegypti* [23]. Replicate assays were conducted on three consecutive days with independently hatched cohorts of larvae. Assays were conducted in 200 μL volumes that including 5 first instar larvae, 10 μL of a 2% food slurry (2:1 pig chow:alfalfa powder), and 2 μL of each compound stock. Negative control DMSO and positive control permethrin were included in each assay. Technical duplicate wells were prepared for each compound and control. Mortality was scored at 24 hours and assays were prepared and maintained at 22˚C throughout the course of the assay. For each biological replicate, the average mortality was calculated from the two technical duplicates. Then, the overall average mortality and standard deviation were calculated from the three biological replicates. PPM values shown in Table 3 are the final concentration of lignas in each well.

3. Results

From the crude extract of *P. cubeba* four major compounds were isolated and were identified as (+)-dihydroclusin (1), (−)-cubebin (2), (−)-clusin (3) and (−)-yatein (4) (Figure 1). Among the isolated compounds, cubebin (26 mg) and clusin (17 mg) presented the highest yields of 10.4% and 6.8%, respectively. Yatein (13 mg) yielded 5.2%, while dihydroclusin (10 mg) had the lowest yield at 4%. The identities of these isolated compounds were confirmed using NMR, MS, and specific rotation measurements, compared with the literature.

All the isolated compounds were evaluated for their herbicidal properties against a dicotyledon (*Lactuca sativa*) and a monocotyledon (*Agrostis stolonifera*) (Table 1). All the compounds showed phytotoxic activity against *A. stolonifera*. Compounds dihydroclusin and clusin showed a rank of 3, when there is 50% inhibition of seed germination, while cubebin and yatein presented lower activity,

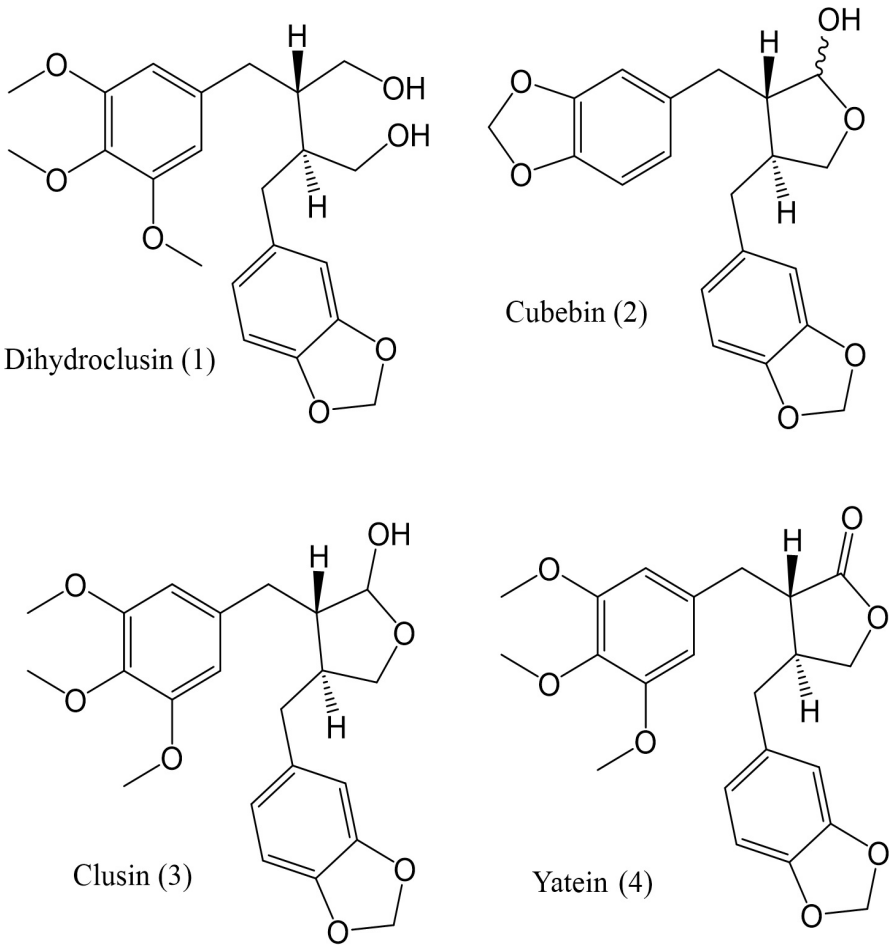

Figure 1. Lignans isolated from *P. cubeba*.

with a ranking of 2, indicating less than 50% seed germination inhibition (**Table 1**). None of the compounds showed activity against *L. sativa*. In comparison, the control, atrazine, presented a ranking of 4 against *A. stolonifera*. These results suggested a selectivity in phytotoxic activity towards monocotyledons by the lignans from *P. cubeba*.

Table 1. Phytotoxicity of atrazine and compounds isolated from *Piper cubeba* seeds. All compounds were tested at 1 mM.

Compounds	Ranking score[a]		*Lemna paucicostata* [IC_{50} (μM)]
	Lactuca sativa	*Agrostis stolonifera*	
Dihydroclusin (1)	0	3*	2.9
Cubebin (2)	0	2	-
Clusin (3)	1	3	45
Yatein (4)	0	2	-
atrazine	3	4	1.45

[a]Ranking based on scale of 0 to 5. 0 = no effect; 5 = no germination; 3 = 50% germination.
*Seedlings grew shorter.

To evaluate the quantitative value of phytotoxic activity, dihydroclusin and clusin were tested on the growth of *Lemna paucicostata* at varying concentrations (Figure 2). Dihydroclusin showed an IC_{50} value of 2.9 μM, while clusin showed an IC_{50} value of 45 μM.

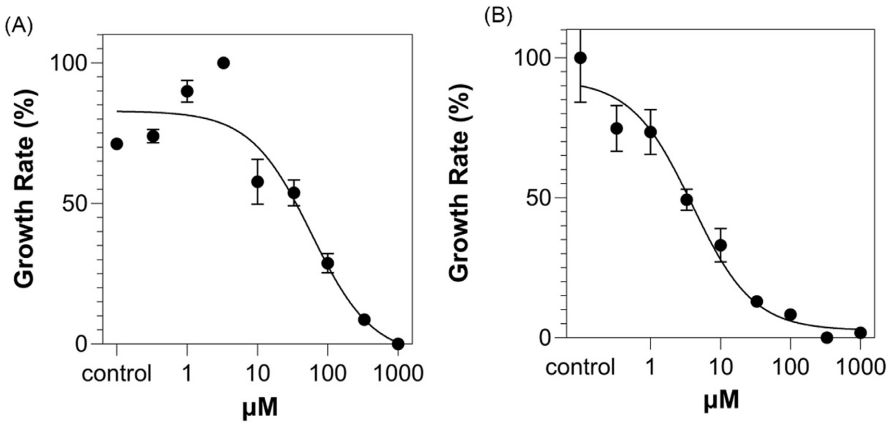

Figure 2. Effects of clusin (A) and dihydroclusin (B) on the growth (%) of duckweed (*L. paucicostata*) at varying concentrations after 7 days of exposure. Error bars are ±1 standard error of the mean.

The isolated compounds were also evaluated for their fungicidal activity using a bioautography assay. Visible fungal growth inhibition zones were observed after incubation, these zones were measured (Table 2). Except for the dihydroclusin, all other lignans compounds exhibited fungicidal activity against strawberry anthracnose pathogen *Colletotrichum fragariae* at 100 μM concentration. Notably, cubebin and yatein showed inhibition of *C. fragariae* at 50 μM.

Table 2. Fungicidal activity of lignans against *Colletotrichum fragariae* in a bioautography assay.

Compounds	inhibitory-zone diameter (mm)			
	100 μM	50 μM	20 μM	10 μM
Dihydroclusin (1)	0	0	0	0
Cubebin (2)	11.1 ± 0.2	6.4 ± 0.4	0	0
Clusin (3)	9.5 ± 0.3	0	0	0
Yatein (4)	15.0 ± 0.4	9.6 ± 0.5	0	0
Solvent control (methanol)	0	0	0	0
Captan	-	-	-	15.7 ± 0.2

Regarding larvicidal activity, dihydroclusin demonstrated activity, causing the mortality of 96.7% of the larvae of *Aedes aegypti* at a concentration of 100 ppm, while cubebin induced mortality of 30% of the larvae at the same concentration. In contrast, clusin and yatein were inactive (Table 3).

Table 3. Larvicidal activity of compounds isolated from *Piper cubeba* seeds.

Compounds	Mortality (% ± SD)	
	100 ppm	20 PPM
Dihydroclusin (1)	96.7 ± 5.8	3.3 ± 5.8
Cubebin (2)	30 ± 5.2	0 ± 0
Clusin (3)	0 ± 0	0 ± 0
Yatein (4)	0 ± 0	0 ± 0
Permethrin (0.008 ppm)	81.7 ± 16.1	
DMSO	0 ± 0	

4. Discussion

The isolation and identification of these types of compounds from the crude extract of *P. cubeba* highlight the phytochemical profile of this plant, mostly composed of lignans. However, other types of compounds have already been isolated and identified, such as *β*-asarone and malonaldehyde [14]. Cubebin is often reported as the most abundant constituent in *P. cubeba* and has been linked to its medicinal properties.

The evaluation of the isolated compounds from *P. cubeba* for their herbicidal properties against *L. sativa* and *A. stolonifera* revealed potential phytotoxic activity. Notably, the isolated compounds exhibited activity only against the monocotyledon *A. stolonifera*. The seeds treated with dihydroclusin exhibited stunted growth, which has significant implications for assessing the phytotoxicity of this compound. The stunted seedling growth suggested that dihydroclusin might interfere with essential physiological processes necessary for plants development. While the exact mechanism of action for dihydroclusin specifically remains to be fully elucidated, these effects could be attributed to a variety of mechanisms, including alterations in nutrient uptake, inhibition of cell division, root system damage or interference with photosynthesis, or inhibition of actin polymerase [21].

When evaluating the effects of clusin on *L. paucicostata*, it is possible to observe that at concentrations below 1 μM, clusin stimulates the growth of *L. paucicostata*. This is a characteristic phenomenon of hormesis, where a substance can have a growth stimulating effect at low doses, while at higher doses can have inhibitory or even toxic effects. This phenomenon is well-documented in some synthetic herbicides, such as 2,4-Dichlorophenoxyacetic acid (2,4-D) [24]. However, hormesis has also been observed in various natural products, such as parthenin, isolated from *Parthenium hysterophorus* [25]. The occurrence of hormesis in plant physiology can trigger responses that influence cellular and molecular mechanisms, including signaling pathways and the activation of antioxidant enzymes, ultimately resulting in increased crop yields [24].

Despite the great potential as bioherbicides, there are few studies evaluating the phytotoxicity of *P. cubeba* compounds. Most research on the phytotoxic activity of

the genus *Piper* focused on the essential oil obtained from the fruits of these plants [26] [27]. However, some studies have demonstrated that certain lignans can have significant phytotoxic effects on weeds and crops of agricultural importance [28]. For instance, some aryltetralin lignans isolated from *Podophyllum peltatum* L. inhibited the growth of dicotyledonous and monocotyledonous plants by interfering with formation of mitotic microtubular organizing centers [29]. Additionally, two furofuran lignans, sesamin and ashantin, from *Artemisia arborescens* inhibited the growth of *A. stolonifera* and *L. sativa* [30]. While lignans showed potential as natural herbicides, further studies are essential to understand the underlying mechanisms of lignan-induced phytotoxicity for the development of sustainable and effective weed management strategies.

The results indicated the fungicidal activity of lignans against the fungal pathogen *C. fragariae*, has significant implications for agricultural disease management strategies. The observed inhibition zones of fungal growth demonstrated the potential of lignans as natural fungicides. These findings align with previous research indicating the antifungal properties of cubebin and other lignans against various fungal pathogens, such as *Penicillium purpurogenum*, *P. madriti* [31], *Aspergillus flavus* [32] among others [12] [31] [32]. Extracts of *P. cubeba* have shown activity against opportunistic oral fungal pathogens *Candida albicans* and *Saccharomyces cerevisiae* [33], and its oleoresins were active against different food pathogenic fungi [31].

Cubebin and some semi-synthetic derivatives were also active against oral fungal pathogens. It was found that the presence of the carbonyl group at C-9, along with the introduction of polar groups in the aromatic rings, improved the antimicrobial activity of the compounds [34]. Our results further support these findings, as the dibenzylbutyrolactone yatein, was more active against *C. fragariae,* inhibiting its growth at a lower concentration compared to the dibenzylbutyrolactol analogs, clusin and cubebein. In contrast, the dibenzylbutyrolactol dihydroclusin was inactive, suggesting that the lactonic ring may have played an important role in the fungicidal activity of these compounds.

The observed differences in larvicidal activity among the tested compounds, dihydroclusin, cubebin, clusin, and yatein, highlight the diverse bioactivity present within the chemical profile of *P. cubeba*. Dihydroclusin exhibited notably high larvicidal activity, indicating that dihydroclusin has potent larvicidal properties, making it a promising candidate for mosquito control. Cubebin displayed lower larvicidal activity at the same concentration. Gomes *et al.* [16] assessed the larvicidal activity of cubebin and hinokinin against *A. aegypti* larvae, revealing only hinokinin's efficacy. Through molecular docking analysis, the proposed mechanism of action was related on the UDP-N-acetylglucosamine pyrophosphorylase protein interaction, crucial for chitin synthesis [16].

The variance in larvicidal activity between dihydroclusin and cubebin could be attributed to differences in their chemical structures, mainly in the lactolic ring. Clusin and yatein were found to be inactive against the tested larvae under the

experimental conditions. This observation suggests that these compounds may not possess significant larvicidal activity against the target species or may require higher concentrations for efficacy.

5. Conclusions

The lignans dihydroclusin, cubebin, clusin, and yatein were successfully isolated from the crude extract of *Piper cubeba*, with cubebin and clusin yielding the highest amounts. The isolated compounds demonstrated selective bioactivity across different assays. In phytotoxicity tests, dihydroclusin and clusin exhibited notable activity against *Lemna paucicostata* indicating their potential as bioherbicides. The hormetic response observed with clusin at low concentrations highlights its complex interaction with plant systems. In fungicidal assays, cubebin and yatein were effective against *Colletotrichum fragariae*, while dihydroclusin's inactivity suggests the structural importance of the lactonic ring for antifungal activity.

In the larvicidal assays, dihydroclusin was the most potent, causing high mortality in *Aedes aegypti* larvae at 100 ppm. Cubebin showed moderate activity, while clusin and yatein were inactive against the larvae. Overall, the study demonstrates that the lignans isolated from *P. cubeba* possess significant herbicidal, fungicidal, and larvicidal properties, positioning them as promising natural alternatives to synthetic pesticides for agricultural pest and disease management.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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