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Original Article

The Efficacy of Crinum Zeylanicum L. Leaf Extract Analysis by GC-MS in Inhibiting Mycelial Growth of Sclerotinia Sclerotiorum Isolates on Common Bean (Phaseolus Vulgaris L.)

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Abstract *- White mold caused by Sclerotinia sclerotiorum is a disease that attacks more than 400 plant species. Controlling it in the field requires the use of chemical fungicides, which are toxic and polluting. This work aimed to estimate the effectiveness of bioactive compounds present in Crinum zeylanicum leaf extracts against Sclerotinia sclerotiorum isolates. Thus, the aqueous, acetone and methanol leaf extracts of C. zeylanicum were obtained, analyzed by GC-MS and tested at different concentrations on mycelial growth and spores germination of S. sclerotiorum isolates. MIC_{50} and MIC_{90} (minimum inhibitory concentrations) were determined. Results showed that plant extracts are rich in phytochemical compounds, with 39, 32, and 10 being methanol, acetone, and aqueous extracts, respectively. Many of these compounds were detected to have biocidal activity. Total inhibition (100%) was obtained at 120 µg/mL with aqueous extract and 60 µg/mL with methanol and acetone extracts. No MIC_{50} could be determined with methanol extracts in both isolates. The lowest inhibitory concentrations were then obtained, with acetone extracts with 1.51 and 0.35 µg/mL for isolates 1 and 2. The highest MIC_{90} were obtained with the aqueous extract (11.39 and 11.53 µg/mL for isolates 1 and 2). Extracts also led to a total inhibition of spore germination from 60 µg/mL with aqueous extract and from 30 µg/mL with acetone and methanol extracts. These results sufficiently demonstrate the richness in active molecules of C. zeylanicum leaf extracts and their huge potential in bean white mold control.*

Keywords *- Crinum zeylanicum, GC-MS analysis, Plant extract, Sclerotinia sclerotiorum.*

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a leguminosae whose domestication would have occurred in two distinct centres: the Mesoamerican region and the Andean region in South America. It was transported to Africa by the Portuguese at the beginning of the 20th century when it was mainly produced in the central and eastern regions [1,2]. Common beans provide nearly 35% and 340 calories/100g in poor population diet; it is therefore called "poor's meat". It also provides important vitamins and minerals such as iron (130 µg/g) and zinc (21-54 µg/g) [3,4,5]. Just like other legumes, beans can fix atmospheric nitrogen thanks to the symbiosis that its roots establish with bacteria of the *Rhizobium* genus, which gives it significant economic and ecological advantages. Common beans are farmed in more than 128 countries; between 2002 and 2021, the area harvested of dry beans has increased by approximately 40% from 27.69 to 35.92 Mt, while total production has augmented from 19.92 to 27.72 Mt, an increase of about 30 % showing the great importance of this crop. In 2021, dry bean yield was estimated at 1.99 t/ha in North America; it was about 2.09 t/ha in Europe and just 0.86 t/ha in Africa (FAOSTAT 2023). This low African yield is due to biotic and abiotic constraints and the absence of resistant varieties or tolerance to these constraints within the primary gene pool of common bean production areas. In Africa, more than 200 pathogens (fungal, bacterial and viral) are known in common beans, and some of them cause considerable economic damage [6]. One of these pathogens is *Sclerotinia sclerotiorum* (Lib.) de Bary, which is responsible for white mold, also known as sclerotiniosis. It is an omnivorous fungus which attacks more than 400 species of plants grouped in more than 270 genera [7]. Symptoms appear during or after flowering; affected tissues dry and turn pale brown; greasy-looking spots appear on all aerial parts of the

plant that are in contact with flowers. Infected tissues take on a cottony white appearance as the fungus grows, and soft mold causes the death of branches [8]. Black, globose to elongated or irregularly shaped sclerotia appear in infected tissues. Branches or the whole plant can die, and yield losses can exceed 50%. Due to sclerotia and its wide host range, it is very difficult to control that disease. Chemical fungicides are often effective, but they have limits, and they are expensive.

In non-developed countries, chemical pesticides are used by farmers in rural areas where the doses are not respected, poor labeling and even the use of expired products [9]. Chemical pesticides are also dangerous for non-target organisms: they have a negative impact on the environment and human health, and there is an immediate need for better strategies in controlling field pests.

One of these alternatives is the use of organic pesticides, which are derivatives of natural materials available in human living environments like microorganisms, plant tissues, animals and some minerals [10]. Plants are the major source of synthesizing natural bioactive products called secondary metabolites, which are grouped under phenolics, terpenes, alkaloids, and glucosinolates.

These metabolites provide them numerous properties in self-defense and medicine [11]. Many plant species have been found to have pesticide potential against several fungi [12,13,14]. Plant pesticides are advantageous because they are biodegradable, less expensive, easy to handle, healthy, and respectful of the environment and human health; they can then safely supplement or even replace harmful chemicals [15]. Amaryllidaceae family is an alkaloid-producing plant taxon; over 630 alkaloids have been reported [16]; the alkaloid group is known for their great properties, such as antitumoral, antimicrobial, antiviral, and cytotoxic.

Alkaloids are not the only phytochemicals present in Amaryllidaceae with antifungal properties. Unfortunately, there is a dearth of information available on the biological properties of other constituents of this family [17,18]. Genus *Crinum*, a member of the Amaryllidaceae family, has huge, beautiful ornamental plants found in tropical and subtropical climates all over the world.

They are used in traditional pharmacopoeia to treat illnesses and disorders. Like many species of its genus, Crinum zeylanicum (Linn.) contains several compounds such as alkaloids, phenolics, flavonoids, fatty acids, and terpenes. *Crinum zeylanicum*, therefore, proves to be an invaluable source of bioactive molecules that can be used in several areas [19,20,21]; unfortunately, there is insufficient information about the antifungal activity of this species. The present work aims to estimate the effectiveness of bioactive compounds present in *Crinum zeylanicum* L. leaf extracts against *Sclerotinia sclerotiorum* (Lib.) de Bary isolates.

2. Material and Method

2.1. Isolation and Identification of Sclerotinia Sclerotiorum

The plant organs (leaves, stems, pods) showing typical symptoms of the disease were collected from two experimental sites: Minkan (3°45′43″ N, 11°31′44″ E) at 725 m altitude for isolate 1 and Obofianga, (3°40′57″ N, 11°50′20″ E) at 591 m altitude for isolate 2. In the laboratory, the plant organs were cut into pieces of 1 to 1.5 cm and sterilized with 1% sodium hypochlorite for 1 minute, then rinsed three times with sterile distilled water and drained using sterile absorbent paper. The pieces of infected organs were deposited in Petri dishes containing PDA medium and incubated at 22 ± 2°C. After 3 days, the mycelium obtained was subjected to successive subcultures until pure cultures were obtained. Identification was made by macroscopic observations (appearance of the mycelium and sclerotia: color, shape) and microscopic (morphological characteristics of the mycelium and spores as well as sclerotia at 10x40 objective) [21].

2.2. Pathogenicity Test

Leaves from healthy common bean plants were collected, washed and sterilized with ethanol (70°), then rinse with sterile distilled water, blotted with sterile filter paper to remove excess water, and air dried. Leaves were placed in Petri dishes containing sterile absorbent paper soaked in distilled water to maintain humidity; mycelial plugs (7 mm diameter) from a 3 day culture of *S. sclerotiorum* were deposited on the adaxial surface of the leaf; Petri dishes were placed in a dark room at 22°C, the control have not to receive the mycelial plug [22].

2.3. Preparation of Crinum Zeylanicum Leaf Extracts

Fresh leaves of *Crinum zeylanicum* were collected in Akom, a village located in Akonolinga (3° 46′ 00″ N; 12° 15′ 00″ E; 669 m altitude). Identification was done at the National Herbarium of Cameroon. The leaves were washed and dried in shade for 3 weeks and powdered using an electric mixer. Five hundred (500g) of leaf powder were weighed and macerated in 2 L of each methanol and acetone for 72 hours. The mixture was stirred with an electric stirrer to maximize extraction. The solution was then filtered with Whatman No.1 filter paper. The filtrates were concentrated in a rotary evaporator (Büchi R200 Rotary Evaporator at 60 °C). The aqueous extract was obtained by macerating 1 kg of fresh leaves paste in 2 liters of distilled water for 12 hours; the macerate was filtered. Extracts were stored in the refrigerator at 4°C [23,24].

2.4. GC - MS Analysis

Crinum zeylanicum leaf extracts (acetone, methanol and aqueous) were analyzed using gas chromatography-mass spectrometry (GC-MS). An Autosystem XL (Agilent GC 7890A) with a split-mode vaporization injector (1:50) and a Perkin-Elmer Turbomass (Aligent Technologies 579 C TAD VL MSD) were used. The HP-5 MS with a capillary column (30 m × 0.25 mm, 0.25µm) was used for sample analysis. The

carrier gas used was helium (99.99%) at a flow rate of 1 mL/min. Injected solutions were prepared for each extract. For the methanol and acetone extract in each tube, 1 mL crude extract was mixed with 2 mL of its extraction solvent, to which 0.15 g of magnesium sulphate was added. For the aqueous extract in one tube, 10 mL were taken and mixed with 10 mL of acetonitrile, to which 6 g of magnesium sulphate and 1.5 g of sodium acetate were added. The tubes were vortexed for 1 minute and placed in a centrifuge at 4000 rpm. From the supernatant, 1 µL of each solution was removed and placed in vials for GC-MS. The injection temperature was 250°C, and the column temperature was as follows: 100°C for 2 minutes, then increased to 200°C at a rate of 10°C per minute, then 5°C per minute up to 280°C. The temperature was maintained at 280°C for 9 minutes. The Electron Impact (EI) was at 280°C, and the ionization energy was 70 eV. Compounds were identified by comparing their mass spectra with those available in the National Institute Standards and Technology (NIST) database and Wiley libraries [25].

2.5. In Vitro Tests

2.5.1. Evaluation of Plant Extracts on Mycelial Growth

A stock (500 µg/mL) was prepared by mixing 50 mg of pure organic extract with 10 mL of extraction solvent in order to dissolve the extract. For organic extracts, 100 mL of distilled water was added. The mixtures were stirred and then left under the hood for 10 minutes to evaporate the extraction solvents. For the aqueous one, the extract was filtered with Whatman No. 1 filter paper and centrifuged. Culture media with respective concentrations of 15, 30, 60 and 120 µg/mL were prepared by mixing respective volumes of 0.9, 1.8, 3.6 and 7.2 mL of the stock solution at 29.1, 28.2; 26.4 and 22.8 mL of PDA for a total volume of 30 mL each.

Ten (10 mL) of each mixture obtained was poured into 90 mm Petri dishes. Negative control was obtained by directly pouring 10 mL of culture medium into the Petri dishes. The positive control, the PDA medium enriched with the fungicide, was obtained by taking 0.1 mL of fungicide (Azoxystrobin 250 mg/L) and mixing it with 29.9 mL of PDA medium for a total volume of 30 mL. Each treatment was inoculated with a 7mm explant loop from dishes containing 4 days pure isolates (1 and 2) of *S. sclerotiorum.* The explant was placed in the center of Petri dishes. Each treatment had three replicates. The mycelial expansion was measured, and the Percentage of Inhibition (PI) of different treatments was calculated 4 days after inoculation with the formula used by [26].

$$PI = \frac{(\mathrm{Dc} - \mathrm{Dt})}{\mathrm{Dc}} \mathrm{x}\ 100$$

The introduction should be succinct, with no subheadings. Limited figures may be included only if they are truly PI: Percentage of inhibition (%); Dc: diameter of the mycelium in the control; Dt: diameter of the mycelium in the treatment.

2.5.2. Determination of the Minimal Inhibition Concentration of the Different Extracts

Minimal Inhibitory Concentrations at 50 and 90% (MIC_{50} and MIC_{90}) of the different extracts with respect to the different extracts were determined 4 days after inoculation using the formula used by España et al. (2017) [27].

$$PI = f(\ln Ci)$$

With PI: percentage of inhibition; Ci: corresponding concentration

To determine the MIC_{50} and MIC_{90}, the linear regression line of type Y = ax + b of the function PI = f (ln Ci) was used [28]. With: Y = percentage of inhibition (%), a = the slope of the line, b = constant and $MIC = e^x$

2.5.3. Evaluation of Fungitoxicity of Crinum Zeylanicum Leaf Extracts.

After inoculating Petri dishes containing culture media enriched with different extracts and at different concentrations, the behavior of the isolates was observed. The concentrations at which the growth of the fungus was completely inhibited were listed. The explants were then removed from their boxes and transferred to new culture media without additives. A treatment was considered fungicidal when the explant put back into culture did not resume growth; it was considered fungistatic when the explant resumed growth [29].

2.5.4. Evaluation of Plant Extracts on Spores Germination

Suspensions were prepared from pure cultures for 10 days. The mycelium was scraped and mixed in 1 mL of sterile distilled water. The suspensions were calibrated at 2 x 104 spores per milliliter using the Mallassez cell [30]. Ten (10 µL) of spores suspension were taken with a micropipette and spread uniformly on the glass slides, having previously received the different culture media with concentrations prepared above. Slides were brought to darkness at 22 ± 2°C. The counting was carried out after 24 hours using a light microscope (objective 10x40). Spores germinated or not were counted with around 20 to 30 spores per microscopic field. Germination rates were obtained using the formula [31].

$$GR = \frac{\mathrm{NG}}{\mathrm{NT}}\ x\ 100$$

Where: GR = Germination rate in % NG = Number of spores germinated NT = Total number of spores examined.

2.6. Statistical Analysis

Data obtained were subjected to an Analysis of Variance (ANOVA) based on the smallest significant value, carried out using R software version 4.3.1.; means were compared using the Duncan test at the 5% threshold; results were considered significant when $P < 0.05$. The graphs presented were produced using the Excel spreadsheet from the Microsoft Office 2013 pack.

3. Results

3.1. Identification and Pathogenicity Test of Sclerotinia Sclerotiorum

Sclerotinia sclerotiorum proved to be a fast-growing fungus, and transplants were carried out every 24 hours. The pure isolates were obtained after 4 subcultures. The Petri dishes presented a flat mycelium made up of very fine filaments of a pale white color (Figure 1A).

At 7 days after incubation, various white forms were observed, which darkened over time to brown and then black (Figure 1B). Microscopic observations of the mycelium showed septate and branched hyphae (Figure 1C). The spores were very small, ellipsoid to fusiform (Figure 1D). The pathogenicity test showed leaves with specific symptoms of *S. sclerotiorum* and confirmed the nature of the fungus (Figure 1E)

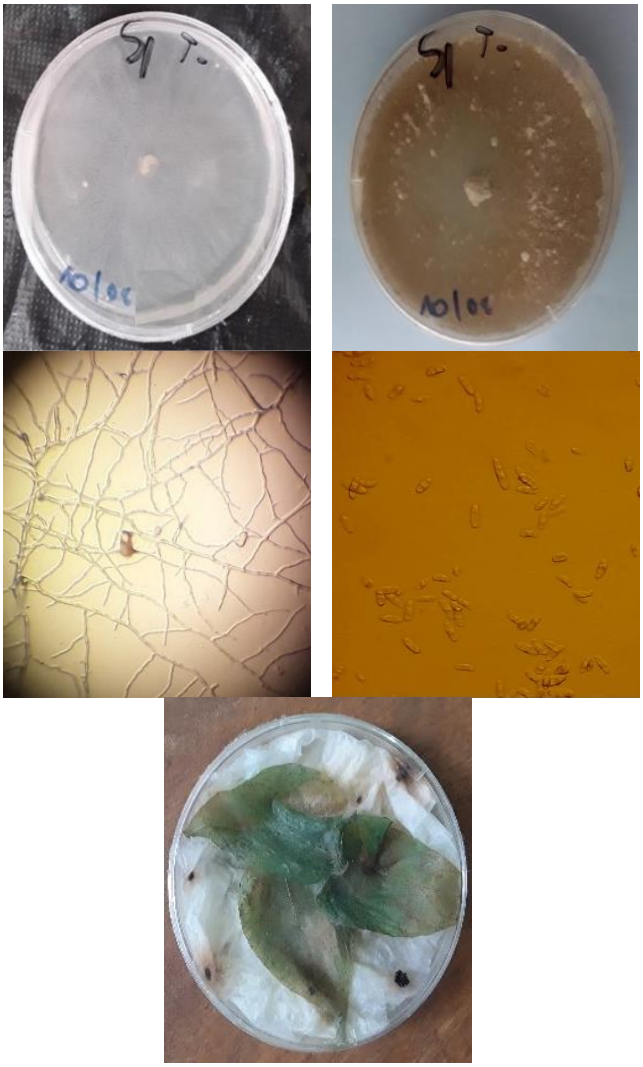


Fig. 1 Isolation and morphological identification of *S. sclerotiorum*
A: Pure isolate 2 days after inoculation, B: Pure isolate 7 days after inoculation, C: Mycelium, D: Spores, E: Bean leave infected 15 days after inoculation

3.2. GC-MS of Crinum Zeylanicum Leaf Extracts

GC-MS of the acetone extract of *C. zeylanicum* leaves reveals the presence of 32 absorption peaks (Fig. 2A). The most abundant were Quinoline-7-carboxylic acid, 2-phenyl-, methyl ester (26.63%); 9,12-Octadecadienoic acid (Z,Z)- (10.83%); 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (10.37%); n-Hexadecanoic acid (7.44%); Daniquidone (5.66%); Phytol (5.27); Benzeneacetic acid, .alpha.-(acetyloxy)-2-methoxy-, methyl ester (3.74%); Hexadecanoic acid, methyl ester (3.66%); 2-Propen-1-one, 1-(4-aminophenyl)-3-phenyl- (2.79%); Benzeneethanamine, N-(1-methylethylidene)- (2.01%). In methanol extract 39 absorption peaks were found (Fig. 2B).

The most abundant were 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (16.89%); Phytol (11.78%); 3-Methyl-12-phenyl-8,9,10,12-tetrahydro-7H-benzo[b][4,7]phenanthrolin-11-one (8.91%); 9-Octadecenoic acid, (E)- (8.28%); n-Hexadecanoic acid (7.94%); Hexadecanoic acid, methyl ester (7.71%); Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)- (5.55%); 11-Hexadecen-1-ol, acetate, (Z)- (4.97%); Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)- (4.3%); Pentadecanoic acid, 14-methyl-, methyl ester (3.69%).

The aqueous extract of *C. zeylanicum* L. leaves showed 10 absorption peaks where, the most abundant of which were n-hexadecanoic acid (35.04%), Cis-Vaccenic Acid (31.76%), .gamma- Sitosterol (7.77%); Octadecanoic acid (6.31%); Hexadecanoic acid, methyl ester (5.37%) (Figure 2C). All the chemical compounds found belong to the family of terpenes, alkaloids, flavoids, phenols, fatty acids; pyrroles and sterols. In these extracts, the significant antifungal activity was due to Hexadecanoic acid, methyl ester, 2-Propen-1-one,1-(4-aminophenyl)-3-phenyl-, 9,12-Octadecadienoic acid (Z-Z)-, methyl ester, Stigmastan-3,5-diene and gamma.-Sitosterol (Figure 3).

3.3. Antifungal Activity of Crinum Zeylanicum Leaf Extracts in Vitro

3.3.1. Effect of Crinum Zeylanicum Leaf Extracts on Mycelial Growth

The results of the percentage inhibition of mycelial growth of *S. sclerotiorum* isolates exposed to different concentrations of the three leaf extracts of *C. zeylanicum* showed that these extracts strongly inhibited mycelial growth independently of the isolates (Figure 4). In fact, 100% inhibition was observed with the three extracts in the same way as with the fungicide treatment.

The aqueous extract causes total inhibition at a concentration of 120 µg/mL, whereas it is observed from a concentration of 60 µg/mL for the other two extracts (acetonic and methanolic). No significant differences ($P<0.05$) were observed between all treatments and fungicides starting with 60 µg/mL at 4 Days After Inoculation (DAI).

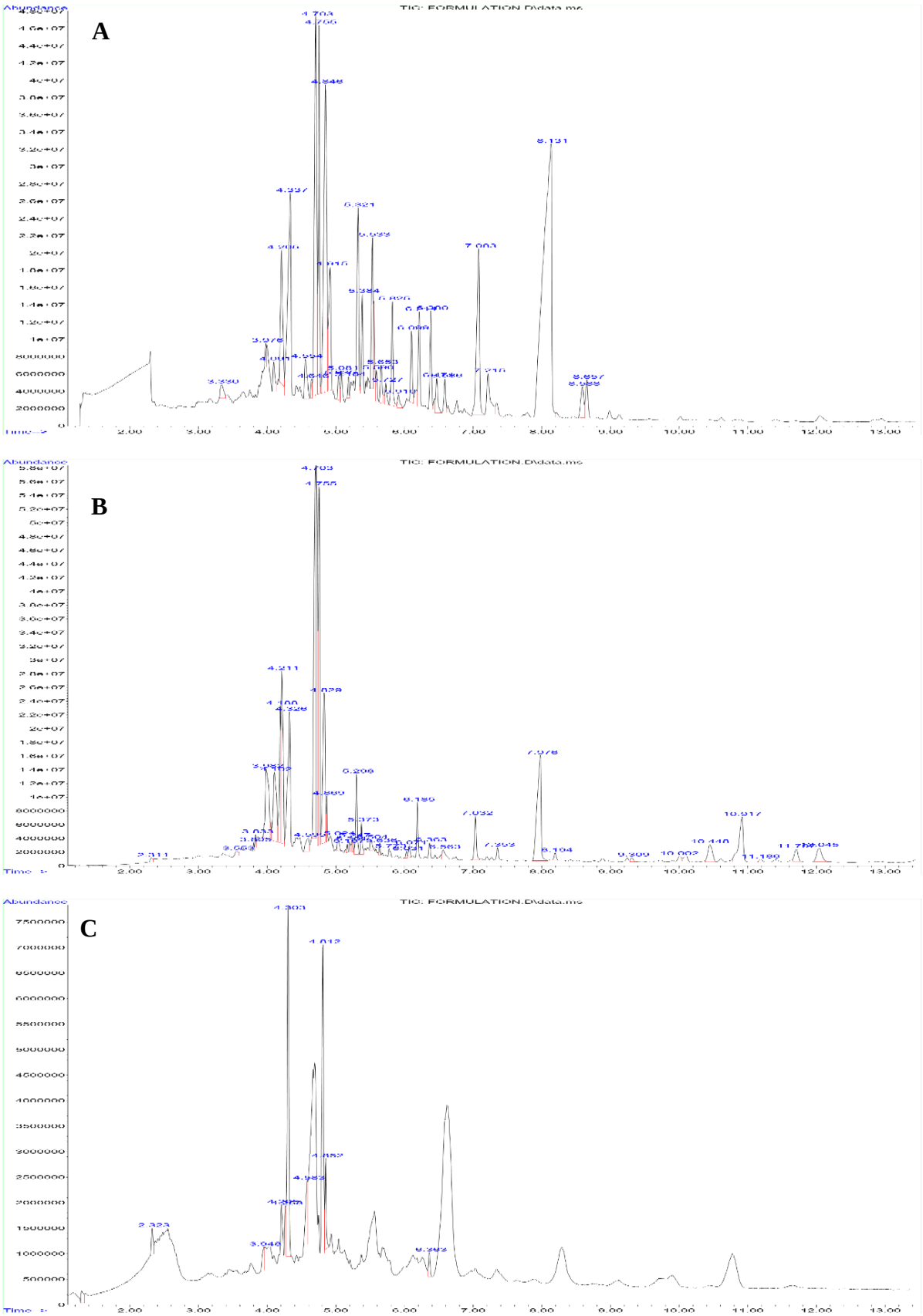


Fig. 2 Chromatographic profile obtained by GC-MS of the acetone extract (A), Methanol extract (B), and Aqueous extract (C) of *C. zeylanicum* leaves

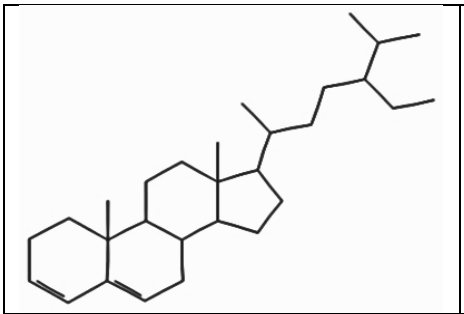
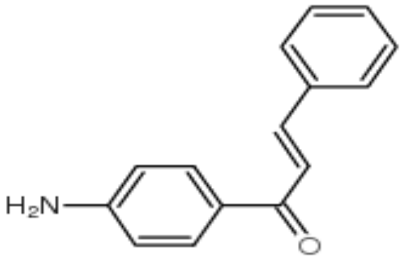
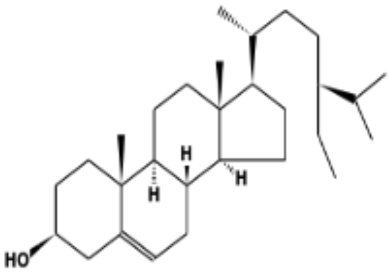
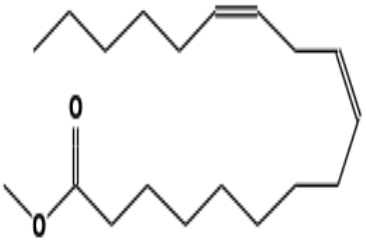
		
Stigmastan-3,5-diene	2-Propen-1-one,1-(4-aminophenyl)-3-phenyl-	gamma.- Sitosterol
		
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Hexadecanoic acid, methyl ester	(-)-trans-Pinane

Fig. 3 Chemical compounds found to have antifungal activity in acetone, methanol and aqueous extract of *C. zeylanicum* leaves.

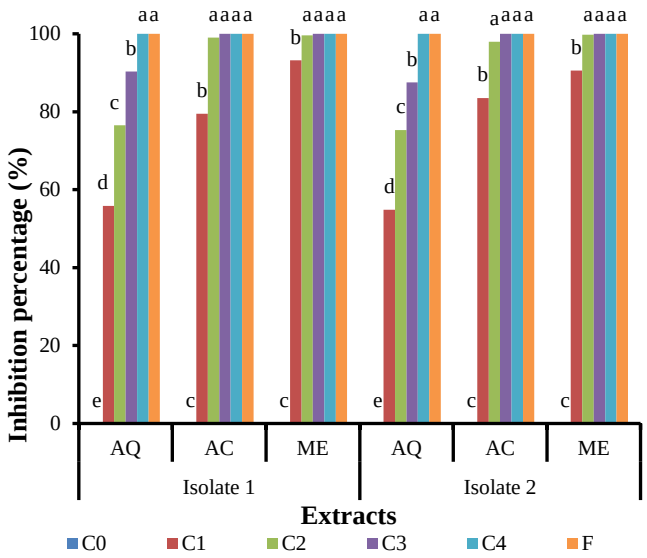


Fig. 4 Percentage of inhibition of mycelial growth at 4 DAI of *Sclerotinia sclerotiorum* isolates (I1 and I2) on PDA media enriched with aqueous extracts (AQ); acetone (AC) and methanol (ME) of *C. zeylanicum* leaves. C0 = control; C1 = 15 µg/mL; C2 = 30 µg/mL; C3 = 60 µg/mL and C4 = 120 µg/mL; F = Fungicide (Azoxystrobin 250 mg/L (3.33 µL/mL)). Bars topped with the same letter in the same treatment are not significantly different (P < 0.05).

3.3.2. Minimal Inhibitory Concentrations MIC_{50} and MIC_{90}

The minimal inhibitory concentrations are presented in Table 1. It shows that the percentages of inhibition were greater than 50% for both isolates. The lowest inhibitory concentrations were obtained after the methanolic extracts, with acetonic extracts at 1.51 and 0.35 µg/mL for isolates 1 and 2. The highest MIC90 was obtained with the aqueous extract (11.39 and 11.53 µg/mL for isolates 1 and 2). No MIC50 could be determined with the methanolic extracts.

3.3.3. Fungitoxycity of Crinum Zeylanicum Leaf Extracts

The fungicidal and fungistatic effect of extracts shows that the concentrations which induced total inhibition of mycelial growth in all treatments were fungicides in both isolates. The other concentrations inhibiting mycelial growth were fungistatic (Table 2).

3.3.4. Effect of Crinum Zeylanicum Leaf Extracts on Pores Germination

The analysis of the results obtained from the study of the effect of *C. zeylanicum* extracts on the spores germination of *S. sclerotiorum* (Figure 5) shows that there is a very significant difference both at the level of the isolates and at the level of treatments; with isolate - treatment interactions. The spores of isolate 1 (I1) were more resistant to the various treatments compared to those of isolate 2 (I2). The negative control (0 µg/mL) allows the germination of *S. sclerotiorum* spores with a rate of 100%. In the acetone extract and the methanol extract, only the concentration of 15 µg/mL was favorable to the germination of spores, with rates of 29 % in isolate 1 and 11 % in isolate 2 for acetone extract and 11.33 % in isolate 1 and 5 % in isolate 2 for the methanol extract. In the aqueous extract, the germination rate decreases with increasing doses of the extract; total inhibition is observed from the concentration of 60 µg/mL for both isolates.

Table 1. MIC_{50} and MIC_{90} (µg/mL)

Isolates	Type of extract	MIC_{50}	MIC_{90}
Isolate 1	**Aqueous**	6.25	11.39
	Acetone	1.51	8.78
	Methanol	*	2.80
Isolate 2	**Aqueous**	6.44	11.53
	Acetone	0.35	8.21
	Methanol	*	5.16

* Represents values that are not set to be zero statistically

Table 2. Fungicidal and fungistatic effect of extracts of extracts

	Aqueous extract		Acetone extracts		Methanol extracts	
	Isolate 1	**Isolate 2**	**Isolate 1**	**Isolate 2**	**Isolate 1**	**Isolate 2**
C2	*	*	Fungistatic	Fungistatic	Fungistatic	Fungistatic
C3	*	*	Fungistatic	Fungistatic	Fungicide	Fungicide
C4	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide

*Represent concentrations which did not inhibit mycelial growth

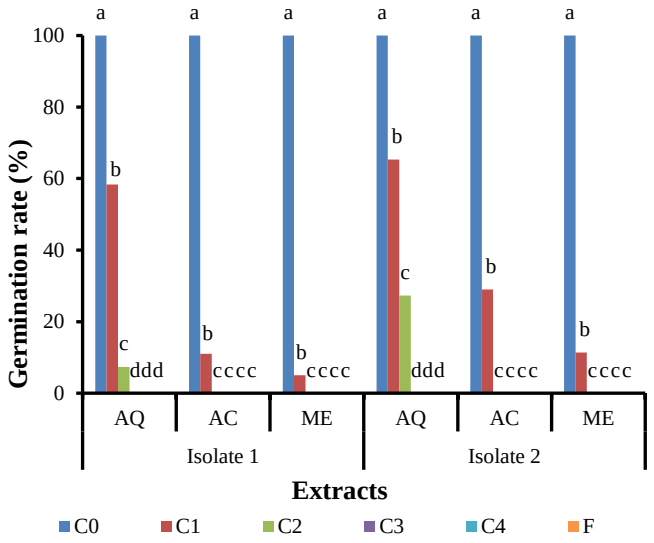


Fig. 5 Germination of *S. sclerotiorum* spores on media enriched with aqueous extracts (AQ), acetone (AC) and methanol (ME) of *C. zeylanicum* leaves. C0 = control; C1 = 15 µg/mL; C2 = 30 µg/mL; C3 = 60 µg/mL and C4 = 120 µg/mL; F = Fungicide (Azoxystrobin 250 mg/l (3.33 µL/mL)). Bars topped with the same letter in the same treatment are not significantly different (P < 0.05).

4. Discussion

This study was carried out on the alternatives to chemical fungicides against *Sclerotinia sclerotiorum.* Aqueous, acetone and methanol leaf extracts of *Crinum zeylanicum* were subjected to GC-MS analysis before *in vitro* test. Morphological observation of *Sclerotinia sclerotiorum* causal agent of bean white mold was made based on macroscopic and microscopic characteristics. It was a fast-growing fungus with a white fluffy mycelium on which masses of various shapes and sizes developed; they were brown, and they were melanized with time: it was sclerotia. In fact, Ordonez-Valencia et al. 2014 and Aldrich-Wolfe et al. 2015 observed that *Sclerotinia sclerotiorum* produced white mycelium and black sclerotia after a few days [32,33]. Pathogenicity test helped to confirm the pathogen. Two days after inoculation, greasy spots were observed. After 7 days, black sclerotia appeared just like described by Tu (1984) [8]. GC-MS of the different extracts of *Crinum zeylanicum* leaves revealed the presence of several chemical compounds. The methanol extract had more peaks, 39 in total. It was directly followed by the acetone extract with 33 absorption peaks and the aqueous extract with 10 absorption peaks. These differences can be explained by the fact that the solubility of a compound in a solvent depends on several factors, including the polarity of the compound and the solvent. Compounds tend to dissolve better in solvents of similar polarity. The polarity of organic solvents (methanol, acetone) would, therefore, be closer to that of the compounds contained in *Crinum zeylanicum* leaves, which enabled them to bind more compounds compared to water, helping to increase the number of molecules extractable by them. Zubair et al. (2013) recommend methanol as a solvent for extracting active ingredients, particularly phenolic compounds [34]. Several compounds of diverse nature and biological functions were identified in the three extracts. This is because *Crinum zeylanicum* belongs to the Amaryllidaceae family, which includes plants that are very rich in bioactive principles [35,36]. Iannello et al. (2014) state that Crinum is, in fact, very rich in phytoconstituents, which have demonstrated significant antibacterial and antifungal activities [37].

The extracts used in this experiment were very effective in inhibiting the mycelial growth of *S. sclerotiorum* isolates. Inhibition percentages of up to 100% were recorded with the three extracts at different concentrations (120 µg/mL for the aqueous extract and 60 µg/mL for the acetone and methanol extracts). Several plants have already demonstrated their efficacy in inhibiting the mycelial growth of fungal pathogens. For example, Wahab et al. (2020) showed that *Moringa oleifera* leaf extracts caused 100% inhibition of in vitro cultures of *Sclerotinia sclerotiorum* and *Botrytis cinerea* [38]. Dias et al. (2019) have also shown that limonene, a

monoterpene extracted from *Citrus* sp. fruit peel, inhibited 100% of mycelial growth of *S. sclerotiorum* at 200 and 300 µL doses [39]. The mechanism of action, as well as the exact molecules to explain the antifungal properties of the extracts, cannot be expressed with accuracy. Many studies nevertheless show that the extracts attack the cell walls of pathogens. The molecules contained in the extracts are associated with polysaccharides and membrane proteins and form complexes with them, which disrupt the fungal plasma membrane and are likely to lead to their lysis and, therefore, the death of the pathogen [40]. Yoshimi et al. (2022) demonstrated that plant extracts rich in phytochemical molecules inhibit the synthesis of β-1,3glucan, an essential polysaccharide in the composition of the cell membrane of several fungi [41]. Phytochemicals thereby interrupt the biosynthesis of the pathogen's cell membrane and, therefore, inhibit the cellular synthesis pathway [42].

The low minimal inhibitory concentrations (MIC_{50} and MIC_{90}) and total inhibition concentrations of germination and mycelial growth show that the phytoconstituents of *C. zeylanicum* leaf extracts act at low doses. Their plurality could explain this. Biomolecules, which vary in nature and concentration, derive their efficacy not from a single action but rather from a synergy between several molecules. This is how Jasso De Rodríguez et al. (2006) asserted that the inhibition potential of plant extracts would result from the molecular composition and concentrations of the bioactive compounds contained in the plant [43].

Research into the fungicidal or fungistatic activity of *C. zeylanicum* leaf extracts revealed that at a concentration of 120 $\mu g.mL^{-1}$ in all the extracts, mycelial growth did not resume on the new culture medium without additives; they were, therefore, all fungicidal-like methanol extract at 60 $\mu g.mL^{-1}$. At the other total inhibition concentrations, extracts were fungistatic. This means that the effectiveness of extracts depends on the concentration of molecules present in them; Kone et al. (2018) found that extracts of Jatropha curcas were more efficient against *Cercospora malayensis* when concentrations increased [44].

Antifungal activity of *C. zeylanicum* extracts could be attributed to the different molecules identified and listed as antimicrobials, such as 9,12-Octadecadienoic acid (Z,Z)-, methyl ester [45]; Octadecanoic acid [46]; Bis(2-ethylhexyl) phthalate [47]; and even antifungals such as Pentadecanoic acid [48]; .gamma.- Sitosterol [49]; Hexadecanoic acid, methyl ester [50]. n-Hexadecanoic acid, which is found in the three extracts, is listed, according to Nepal et al. (2021), is a pesticide [51]. Other molecules such as Tetradecanoic acid, 12-methyl-, methyl ester; Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1S-(1.alpha.,2.beta.,5.alpha.)]- and 2-Methyl-Z,Z-3 ,13-octadecadienol are also listed as pesticides [52,53,54]. The extracts used were also shown to be very effective in inhibiting the germination of *S. sclerotiorum* spores. Germination rates of 0% were obtained with small concentrations of extracts. This effectiveness can be attributed to the fact that chemical compounds present in the different extracts, such as antioxidants, can interfere with cellular metabolism; they can disrupt essential metabolic pathways in spores, inhibiting their ability to germinate.

For example, they can inhibit the synthesis of enzymes required for glycolysis or cellular respiration at nuclear or ribosomal levels, thereby blocking the energy needed for germination; these secondary metabolites can also substitute the limiting factor in intermediary metabolism [55]. Several plant extracts have already shown themselves to be very effective in inhibiting the germination of fungal pathogen spores.

This is how Achraf et al. (2012) showed that aqueous extracts of *Asphodelus tenuifolius* and *Zygophyllum album* inhibit the germination of *Penicillium expansum* spores with inhibition percentages of 95.48% and 93.82% [56]. Okigbo and Ogbonnaya (2006) also showed that ethanolic extracts of the leaves of *Ocimum gratissimum* and *Aframomum melegueta* inhibit the germination of *Fusarium oxysporum* and *Aspergillus niger* spores by more than 65% [57].

5. Conclusion

The GC-MS analysis of methanol, acetone, and aqueous extracts of Crinum zeylanicum leaves made it possible to obtain several molecules of a varied nature that presented diverse biological activities. The results obtained sufficiently demonstrate the richness in active molecules of these extracts, which are responsible for the antifungal activity against isolates of *Sclerotinia sclerotiorum*. The availability of plant material in many ecosystems and the immense potential of this plant can, therefore, be very useful in *S. sclerotiorum* control. Further studies can be carried out to test the effectiveness of this approach in the field.

Authors' Contribution

Ndongo B., Conceptualization; Tsogo S.C. Original draft preparation and data analysis; Ngatsi P.Z., Writing –Review-Editing; Kuate W.N.T., Methodology

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