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Antifungal Activity of *Annona muricata* Seed Extracts Against *Cercospora malayensis*, Causal Agent of Cercospora Leaf Spot Disease of Okra (*Abelmoschus esculentus* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author BN selected the scope of the work and editing the manuscript. Authors WNTK, SLLD, CSE and HB identified diseases and conduct the lab experiment. Author HB write the first draft of the manuscript. Author PZN analyzed data, reviewed and edited the manuscript. Author LBT reviewed, edited and made a major contribution to the final version of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cercospora leaf spot disease of okra whose pathogen is *Cercospora malayensis* causes yield losses of up to 60% in plantations. To limit productivity losses, fungicides are commonly used, but are expensive and degrade the environment.
Aims: This study aims to test in vitro efficacy of *Annona muricata* seed extracts against *Cercospora malayensis*.

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Study Design: Four extracts were used in this study (the ethyl acetate, acetone, methanol and aqueous extract of *A. muricata* seeds at the concentrations C1 = 7.5 µl/ml, C2 = 15 µl/ml, C3 = 30 µl/ml and C4 = 60 µl/ml as well as the synthetic fungicide at the concentration of 3.33 g/l) in triplicate. The phytochemical screening of the extracts was performed, the radial growth of pure explants (7 mm diameter) of *C. malayensis* deposited in sterile Petri dishes containing the PDA medium supplemented with the different concentrations of extracts and incubated at 23 ± 1°C for 6 days were evaluated. Minimum inhibitory concentrations (MIC50, MIC90) were calculated.
Results: The extracts of *A. muricata* seeds are rich in tannins, flavonoids, terpenoids and phenols. The ethyl acetate extract at the concentration C3 resulted in 100% total inhibition of growth of *C. malayensis* in the Petri dishes. The other extracts resulted in total inhibition of the growth of *C. malayensis* at C4. The low MIC50 values (12.9 and 21 µl/ml) were obtained with the ethyl acetate and acetone extract, respectively. The ethyl acetate and aqueous extract at the C4 concentration were found to be fungicidal.
Conclusion: The extracts were found to be potential fungicide against the *C. malayensis* strain and might be an alternative in the fight against fungal diseases of okra as their activity was comparable to that of the synthetic fungicide Monchamp 72 WP.

Keywords: Cercospora malayensis; extracts; Annona muricata; okra; antifungal; growth.

1. INTRODUCTION

The okra (*Abelmoschus esculentus* [L.] Moench) belongs to the family Malvaceae and the genus *Abelmoschus* Med [1]. It is one of the most important and widely cultivated vegetables in terms of surface area and quantities produced in most tropical, subtropical and Mediterranean countries [2] Okra is of considerable economic importance and plays an essential role in the nutritional balance of populations. The originality of okra lies in the fact that all its organs are of interest in terms of food and industrial valorization [3,4].

In 2019, the world production of okra was estimated at 9 million tons. Africa produces about 3.5 million tons and Cameroon 104,216 tons for an area of 24,004 hectares [5]. This low national production since okra is cultivated in very small areas to which are added pests and diseases; in particular, Cercospora leaf spot disease is one of the major diseases of okra caused by *Cercospora malayensis*. It causes damage to the leaves and can cause a loss of yield of more than 60% in the absence of appropriate protective measures. This loss of yield has a remarkable impact on farmers' incomes and food security [6]. Cercospora leaf spot disease has been observed in tropical and sub-tropical Asia and is present in Africa where okra is grown during the rainy seasons [7,8]. Symptoms observed on okra leaves are generally irregular, brown and then turn reddish-brown with a yellowish margin. These symptoms appear on the older lower leaves and progress with new lesions on the younger upper leaves [9]. The use of improved varieties and synthetic chemical pesticides are the means of control used against this pathogenic fungus [10]. However, these inputs are still not available to farmers and chemical pesticides have harmful effects on the health of populations and the environment [11,12].

Numerous studies have been carried out to minimize the use of chemical pesticides and promote the use of plant-based biocides [13]. In this new world concerned about the health of producers and consumers and the preservation of ecosystem balance, the ideal would be that the pesticides of the future are natural products that are biodegradable and capable of interfering directly or indirectly with the metabolism of pests [14]. The use of plant extracts rich in secondary metabolites (phenolic compounds, terpenoids and nitrogen compounds) for their pesticide properties as a means of controlling crop diseases and pests have already successfully demonstrated their effectiveness. Several works have shown the fungicidal effect of *Jatropha curcas* seeds [15,16], the antifungal [17,18,19] and insecticidal [20] effects of *Thevetia peruviana* seeds. Like most biodegradable pesticide products, *Annona muricata* seeds have been the subject of numerous studies that have demonstrated insecticidal, fungicidal, microbial and bactericidal properties [21,22,23,16,24]. Further research efforts are needed to explore the fungicidal potential of *A. muricata* extracts in the control of these plant pathogens. This study proposes to find an alternative to chemical control through the use of *A. muricata* seed extracts. The objective of this work is

to test *in vitro* efficacy of *Annona muricata* seed extracts vis-à-vis *Cercospora malayensis*.

2. MATERIALS AND METHODS

2.1 Biological and Chemical Materials

The plant of *Annona muricata* was identified according to the botanical systematics key of the species by referring to the recent version of the International Code of Botanical Nomenclature [25] and the mature fruits were reported to the National Herbarium for confirmation. The mature fruits were collected in the locality of Manjo belonging to agro-ecological zone 4 with single-modal rainfall (N 04°51'00' and E 09°49'00'). The leaves of okra bearing the symptoms of Cercospora were taken from infected plants in fields free of any phytosanitary treatment, collected in the locality of Akololinga belonging to agro-ecological zone 5 with bimodal rainfall (N 03°48. 136' and E 012°15.518') were also used. The chemical material consisted of the synthetic fungicide Monchamp 72 WP with the active ingredient Metalaxyl 80 g/kg and Mancozebe 640 g/kg, a systemic and contact fungicide commonly used in the control of fungi, and organic solvents (ethyl acetate, methanol and acetone) which allowed the production of the different extracts of *A. Muricata* seeds.

2.2 Methods

2.2.1 Culture medium

The preparation of 1 liter of Potato Dextrose Agar (PDA) culture medium was made using 200 g potato, 15 g agar and 15 g dextrose. The resulting solution was autoclaved for 20 min at 120°C, pressure 1 bar and stored in the refrigerator.

2.2.2 Preparation of extracts of *Annona muricata* seeds

The mature fruits of *A. muricata* were removed from the pulp and the resulting seeds were dried at room temperature for two to three weeks to prevent the development of fungi. Once dry, the seeds were finely crushed using a hand mill and the resulting powder was used to prepare the extracts.

The organic solution of *A. muricata* was made according to the process outlined by Stoll [26]. Using the precision balance (SCALTEC SPB55 with a precision of 0.01 g), 500 g of seed powder was weighed and macerated in 2 liters of solvent represented here by acetone, methanol and ethyl acetate for 72 hours. After filtration with filter paper, the solution was concentrated using a rota-vapor. The different extracts obtained with ethyl acetate (EAE), methanol (ME) and acetone (AE) were weighed and then stored in a cool place at 4°C until use.

The aqueous solution of *A. muricata* was made according to the process used by Ondoa [27]. One hundred gram (100 g) of seed powder was weighed using the balance (SCALTEC SPB 55, precision 0.01 g) and introduced into a container containing 1 liter of distilled water, macerated for 24 hours and filtered with a muslin cloth. The aqueous extract (AqE) obtained was ready for use.

2.2.3 Obtaining the different doses of extracts

To obtain the concentrations of 7.5; 15; 30 and 60 µl/ml, a stock solution of 500 µl/ml was previously prepared for the organic extracts by mixing 10 ml of pure extract with 3 ml of sterile distilled water and 7 ml of 70° ethyl alcohol. For the AqE, a volume of 200 ml was taken from the stock solution. The culture media were prepared by successively taking 0.45, 0.9, 1.8 and 3.6 ml of this solution and adding 29.55, 29.1, 28.2 and 26.4 ml of PDA, respectively, for a final volume of 30 ml each.

The medium enriched with the synthetic fungicide Monchamp 72 WP (F) was prepared according to the manufacturer's recommended dosage of approximately 3.33 g/l. For this purpose, a stock solution of the fungicide (3.33 mg/ml) was previously prepared by introducing 50 mg of powder in sterile distilled water, for a final volume of 15 ml. A volume of 2 ml is then taken from this stock solution and mixed with 28 ml PDA medium for a final volume of 30 ml.

2.2.4 Determination of extraction yields

Extract yields were calculated according to the formula used by Ngho Dooh et al. [28].

Yield (%) = (Mass of extract (g)) / (Mass of powder (g)) x 100

The mass of the extract corresponds to the mass of the liquid obtained after the extraction; the mass of the powder corresponds to the mass of the crushed seeds.

2.2.5 Phytochemical screening

The classes of secondary metabolites present in organic and aqueous extracts of *A. muricata* seeds were determined from standard protocols used by Harbone [29]; Edeoga et al. [30]; Tiwari et al. [31]; Banu and Catherine [32]. These techniques are based on the turbidity, precipitation, and foaming of extracts in the presence of different reagents characterizing each class of secondary metabolites. A volume of 2 ml of aqueous and organic extract of *A. muricata* seeds was used to qualitatively determine the presence of the classes of secondary compounds.

2.2.6 Isolation and purification of the fungus

The infected leaves brought back to the laboratory were cut into fragments of about 2 cm^2 at the growth front of the pathogen and superficially disinfected in a 5% sodium hypochlorite solution for 2 minutes. After two rinses with sterilized distilled water, the fragments were dried on hydrophilic paper and then placed in a Petri dish containing the PDA culture medium supplemented with a solution of antibiotics consisting of penicillin (250 mg/l), ampicillin (250 mg/l) and nystatin (20 mg/l) [33,34], sealed with film and incubated at 22-24°C. The mycelium develops from the leaf fragments and after 5 days reaches sufficient growth to proceed to its purification. Purification was performed by successive transplantation of an explant taken from the mycelium growth front on PDA medium. This operation was repeated 3 times until pure cultures are obtained [35,36]. Spore identification was done using microscopic observations of the conidia and an identification key [37,38].

2.2.7 Evaluation of mycelial growth of *Cercospora malayensis*

Mycelial explants of *C. malayensis* with a the diameter of about 7 mm was collected and deposited in the centre of the Petri dishes containing the medium enriched with the different extracts at concentrations of 7.5; 15; 30 and 60 µl/ml and synthetic fungicide (3.33g /ml). A negative control not supplemented with extract or fungicide was developed. Each treatment was repeated 3 times. Incubation was performed at 23 ± 1°C. The mycelial growth of *C. malayensis* was calculated by measuring two perpendicular diameters drawn on the back of the Petri dishes daily from 2 to 6 days after incubation (JAI) according to the formula used by Singh et al. [39].

$$D = \frac{(d1 + d2)}{2} - d0$$

Where: D = radial growth; d1 and d2 = diameters of the culture measured in the two perpendicular directions; d0 = diameter of the explant.

2.2.8 Fungicidal or fungistatic test of *Annona muricata* extracts

The test consists of evaluating the effectiveness of extracts that have a total inhibition on cultivated *Cercospora malayensis*. *C. malayensis* explants taken from the Petri dishes containing the extract at different concentrations were deposited in new dishes containing the PDA medium. If growth is resumed in the new medium, the extract is qualified as fungistatic; otherwise, it is qualified as a fungicide [40,41].

2.2.9 Determination of minimal inhibitory concentrations of the different extracts

The minimum concentrations inhibiting 50% and 90% (MIC50 and MIC90) the growth of *C. malayensis* were determined by the method of Dohou et al. [42] and by comparing the values of the percentage of inhibition (PI) with those of the Naperian logarithm of the corresponding concentrations (Ci):

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

The percentage inhibition (PI) is determined for each treatment compared to the control after 6 days of growth, according to the formula of Singh et al. [39]:

$$PI\ (\%) = (Dc - Dx)/Dc \times 100$$

Where: Dc = Average culture diameter measured without extract; Dx = Average culture the diameter measured with the extract.

The linear regression line Y = ax + b from the function PI = f (ln Ci) was used to determine the MIC50 and MIC90, where Y = percentage inhibition, a = slope of the line, MIC50 = ex and b = constant.

2.3 Statistical Analyses

The collected data were entered into the Excel spreadsheet for a minimum of three replicates (n=3). One-way analysis of variance (ANOVA) was performed using R software version 3.5.1. The differences between the means were compared by the Tukey test ($P < 0.05$) when differences were recorded.

3. RESULTS

3.1 Extraction Yield

The use of organic solvents (methanol, ethyl acetate and acetone) has made it possible to obtain extracts of *A. muricata* seeds of variable volume and appearance (Table 1). The result obtained shows that the highest yield is obtained with acetone (39.8%), followed by ethyl acetate (38.02%). Extraction with methanol gave the lowest yield (26.02%).

3.2 Phytochemical Screening

Phytochemical screening of the different extracts of *Annona muricata* seeds revealed the presence of several compounds belonging to various chemical classes. Alkaloids, terpenes, coumarins, sterols, phenols, flavonoids, oils, sugars, saponins and tannins are present in the extracts. Alkaloids, flavonoids, sterols and terpenes are the most abundant. Methanol and aqueous extracts are the richest in compounds. The extracts with acetone and ethyl acetate are the poorest in a class of chemical compounds (Table 2).

3.3 Effect of *Annona muricata* Seed Extracts on Radial Growth

The evolution of mycelial growth of *C. malayensis* under the control of aqueous and organic extracts varies according to the concentration used and the control whose mycelial growth fills the Petri dish 6 days after incubation (DAI) (Fig. 1).

At 6 DAI ($P < 0.05$), the aqueous extract (AqE) resulted in radial growth of 5.01, 4.62, 2.2 and 0 cm in diameter at concentrations C1, C2, C3 and C4 respectively. Concentration C3 of the extract with acetone (AE) (1.25 cm), methanol (ME) (0.56 cm) and ethyl acetate (EAE) (0.16 cm) resulted in inhibition of the growth of *C. malayensis* close to the C4 concentration and fungicide; which totally inhibited the mycelial growth of the pathogen (Fig. 2).

Table 1. Yield (%) and characteristics of extracts obtained with 500 g of seed powder

Extracts	Yield (%)	Aspect	Color
AE	39.8	Oily	blackish
EAE	38.2	Oily	blackish
ME	26.02	Oily	blackish
AqE	29.32	Liquid	colorless

AE, acetone extract; EAE, ethyl acetate extract; ME, methanol extract; AqE, aqueous extract

Table 2. Different natural products in the different extracts of *Annona muricata*

Components	EAE	AE	ME	AqE
Oil	+	+	+	+
Coumarins	+	+	+	-
Alkaloids	+	++	+	++
Sterols	+	+	++	+
Terpenoids	+	+	++	++
Flavonoids	+	+	++	++
Tannins	-	-	+	+++
Saponins	+	-	+	+
Sugars	+	+	T	T
phenols	+	+	++	++
Carbonihydrate	+	-	+	++

-, Absence; +, presence; +++, abundant presence; T, trace; AE, acetone extract; EAE, ethyl acetate extract; ME, methanol extract; AqE, aqueous extract

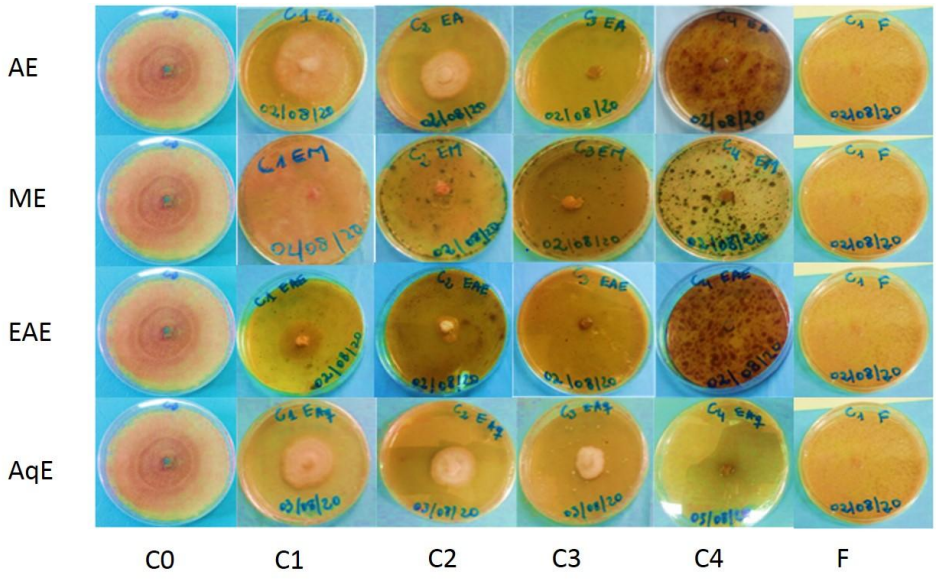

Fig. 1. Inhibition of mycelial growth of *Cercospora malayensis* by extracts of *Annona muricata* seeds at different concentrations. C0 = 0µl/ml; C1= 7.5 µl/ml; C2= 15 µl/ml; C3= 30µl/ml; C4= 60 µl/ml; F= fungicide. AE, acetone extract; EAE, ethyl acetate extract; ME, methanol extract; AqE, aqueous extract

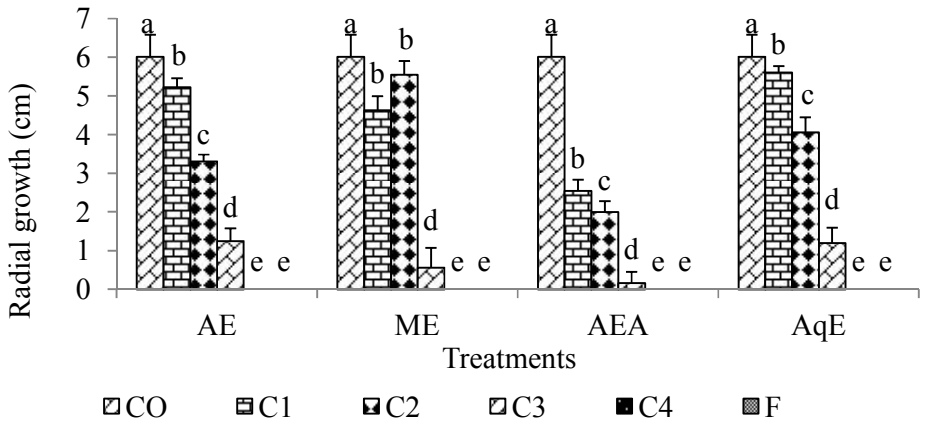

Fig. 2. Evolution of strain diameters under the effect of seed extracts of *Annona muricata.* AE, acetone extract; ME, methanol extract; EAE, ethyl acetate extract; AqE, aqueous extract; C0, 0µl/ml; C1, 7.5 µl/ml; C2, 15 µl/ml; C3, 30 µl/ml; C4, 60 µl/ml; F, fungicide. For each extract, bars with different letters are significantly different at *P < 0.05*

3.4 Fungicidal or Fungistatic Activity of the Extracts

The data in Table 3 present the antifungal status of *A. muricata* seed extracts and fungicide concerning *C. malayensis*. The extracts tested were found to be fungicidal (EAE and AqE) on the one hand, and fungistatic (AE and ME) on the other hand.

3.5 Correlation Test Between the Concentrations and the Percentages of Inhibition Obtained with the Extracts

This test was performed to see if there is a linear relationship between the decrease or increase in inhibition with different concentrations of organic and aqueous extracts on the radial growth of *C. malayensis.* The regression lines obtained after

analysis revealed similar behaviour of *C. malayensis* towards the extracts (organic and aqueous). It appears that all lines obtained show positive slopes and perfect correlations between concentrations and different percentages of inhibition (Fig. 3).

The equations obtained with the different extracts tested show increasing linear relationships with positive slope regression lines: y = 51.35x - 107.79; y = 22.77x + 10.48; y = 42.54x - 70.57; y = 45.53x - 91.62, respectively for the ME, EAE, AE and AqE. A perfect and positive correlation was obtained between the different concentrations and the percentage of inhibition. The correlation coefficient (r^2) was between 0.7 and 1, i.e. r = 0.84; r = 0.99; r = 0.96; r = 0.95 respectively for ME, AE, AqE and EAE (Table 4).

Table 3. Antifungal activity of Annona muricata seed extracts. AE, acetone extract; ME, methanol extract; EAE, ethyl acetate extract; AqE, aqueous extract; C3, 30µl/ml; C4, 60 µl/ml

Extracts	Lethal Concentration	Effect
EAE	C3	Fungicidal
EAE	C4	Fungicidal
AE	C4	Fungistatic
AqE	C4	Fungicidal
ME	C4	Fungistatic

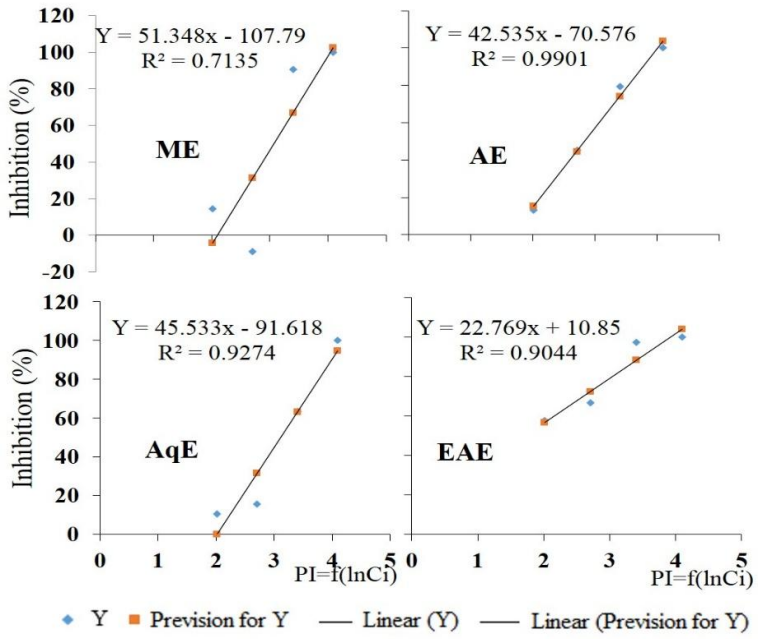

Fig. 3. Regression lines of mycelial growth at different treatments. ME, methanol extract; EAE, ethyl acetate extract; AE, acetone extract; AqE, aqueous extract

Table 4. Correlation between percentage inhibition and concentrations of different extracts on *Cercospora malayensis* strain. ME, methanol extract; EAE, ethyl acetate extract; AE, acetone extract; AqE, aqueous extract

Extracts	Correlation coefficient (r)	Observations
EAE	0.95	Highly correlated
AE	0.99	Highly correlated
AqE	0.96	Highly correlated
ME	0.84	Highly correlated

3.6 Minimal Inhibitory Concentrations of the Different Extracts

The concentrations of the different extracts inhibiting the growth of the fungus by 50% and 90% (MIC50 and MIC90) were determined from the regression lines obtained after the correlation tests (Table 5). The lowest inhibitory concentrations MIC50 and MIC90 were obtained with EAE at 12.9 and 80.4 µl/ml respectively. The highest MIC50 and MIC90 were obtained with the ME at 92.18 and 115.55 µl/ml respectively.

4. DISCUSSION

The extraction of 500 g of *A. muricata* seeds produced different yields. These yields varied according to the solvents used, 39.08% with the AE; 38.02% with EAE; 26.02% with the ME and 29.32% with the AqE. These different yields obtained can be attributed to the nature of the solvent. The difference in yield obtained between the aqueous and organic extract could be explained by the fact that organic solvents fix more compounds compared to water and therefore increase the extraction yield. Tsopmbeng et al. [43] reported the similarly extraction yield. Furthermore, according to Muhammad et al. [44] methanol with its high polarity allows more efficient extraction of secondary metabolites. This difference could also be attributed to the extrinsic factors of the plant, the plant species and/or the organ under consideration. Indeed, Bruneton [45]; Smallfield [46] have reported that atmospheric conditions, the state of the plant material at the time of harvest, the harvest period and the age of the plant material can influence extraction yields. Besides, plant species do not all have the same composition; some botanical families offer higher yields than others [47].

The results of the screening carried out showed the presence of several classes of compounds that are natural bioactive substances such as essential oils, coumarins, sterols, saponins, sugars, terpenes and flavonoids. Several of these compounds have also been obtained by Omolara et al. [48]; Naik and Sellappan [49] with *Annona muricata*.

The aqueous and organic extracts significantly reduced the radial growth of *C. malayensis* compared to the control. This reduction was more pronounced with the organic extracts than with the AqE. Total inhibition of 100% growth was observed for all extracts tested on *C. malayensis* at the concentration of 60 µl/ml. However, EAE was more effective with an inhibition rate of around 100% at the concentration of 30 µl /ml. These extracts contain substances that inhibit or delay the growth of the fungus. Indeed, Pamo et al. [50]; Ngoh Dooh et al. [28] reported that extracts of certain plants contain tannins, flavonoids and alkaloids that have fungicidal properties.

The different concentrations of extracts significantly influenced the radial growth of the fungus; the highest concentrations were the inhibitor with a better behaviour of the organic extracts to the aqueous extract. These results are in line with those reported by Tsompbeng et al. [43] who obtained very high inhibition percentages with the methanolic extracts of *Laggera pterodonta* and *Cupressus lusitanica*, on *Phytophthora colocasiae*. These results are contrary to those of Kone [51], who working on the effect of aqueous and organic extracts of *Jatropha curcas* seeds against *C. maleyensis,* showed that aqueous extracts had a more inhibitory action than organic extracts. On the other hand, Bautista et al. [52] using aqueous extracts from papaya leaves and seeds did not obtain any inhibition of the growth of *Collototrichum gloeosporiodes*. This could be since the chemical composition of the plant extracts could vary according to the nature of the plants and also according to the organ used. Reddy [53] obtained a reduction in the growth of several fungi of the genus *Aspergilus* and *Penicillium* with alcoholic extracts from the leaves of *Thevetia peruviana*. On the other hand, extracts with diclomethane and methanol from the leaves of *Thevetia peruviana* inhibited the growth of *Cladosporium cucumerinum* as shown by the work of Gata-Goncalves et al. [54].

The efficacy of the extracts on the growth of *Cercospora malayensis* could be explained by the presence in these extracts of the bioactive molecules revealed by phytochemical screening, such as curcine and lectin; in addition to these proteins, the presence of secondary metabolites such as phenols, phorbol esters, saponins would be responsible for the antifungal potential of *A. muricata* seed extracts. Zirihi et al. [55] obtained a total inhibition of the mycelial growth of *Pythium aphanidermatum* with aqueous and organic extracts of *Combretum racemosum* for

Table 5. Minimum concentration inhibiting mycelial growth of *Cercospora malayensis* by the extracts tested. EAE, ethyl acetate extract; AE, acetone extract; AqE, aqueous extract; ME, methanol extract

Extraits	MIC50 (µl/ml)	MCI90 (µl/ml)
EAE	12,9	80.4
AE	21	93.1
AqE	93.3	109.99
ME	92.18	115.55

concentrations higher than 6 g/l. Similarly, Djeugap et al. [14] using extracts of *Callistemon viminalis* and *Eucalyptus saligna* on *Phytophthora infestans,* the causal agent of late blight in black nightshade and potato, obtained total inhibition.

The various antifungal tests carried out with aqueous and organic extracts of *A. muricata* were found to be fungistatic (Acetone and Methanol) on the one hand and fungicidal (ethyl acetate and aqueous) on the other hand. These results are contrary to those of Nchare, [56] who obtained fungicidal activity with organic extracts (Acetone, Ethyl acetate, Methanol and Hexane) of *Jatropha curcas* seeds against *Phytophthora megakarya*. This difference in antifungal activity obtained with plant extracts on pathogen strains could be explained by the fact that each phytopathogenic fungus has its genetic characteristics and therefore does not react in the same way to biopesticides. Such results were obtained by Carlton et al. [57] who showed that plant pathogenic fungi act differently in the presence of biopesticides.

All the extracts tested obtained a 100% inhibition of the mycelial growth of *Cercospora malayensis* at C4 concentration which are a similar effect to the fungicide Monchamp 72 WP. The effectiveness of the fungicide would be due to the presence of Metalaxyl, the major active ingredient (80%), which is known for its action on cellular respiration [51].

The correlation tests carried out between the concentrations used and the percentage of inhibition allowed linear relationships between them to be established. The correlation coefficients determined showed that the concentrations of the extracts and the percentages of inhibition are strongly correlated. This body of knowledge makes it possible to understand the degree of dependence between the different parameters tested. When the extract concentration increases; the percentage of inhibition increases and vice versa. In other words, the percentage of inhibition is strictly proportional to the different extract concentrations used. This phenomenon observed in the case of extracts from *A. muricata* seeds show that there was no antagonism or blockade in the increase of extract concentrations on the inhibition of fungal growth. These results confirm those obtained by Soro et al. [58] working on the effect of the extract of powder and essential oil of *Xylopia aethiopica* against *Fusarium oxysporum* silver causal of Fusarium head blight of tomato.

The percentages of inhibitions obtained with certain extracts with inhibitory action and the fungicide (Monchamp) do not show any difference. In other words, these extracts at high concentrations are as effective as the chemical fungicide. Mboussi et al. [18] showed the effect of extracts of *Thevetia peruviana*, *Azadirachta indica* and Ridomil Gold Plus on the *Phytophthora megakarya* strain. Similarly, Ndogho et al. [59] showed inhibition proportional to the concentrations tested using aqueous extracts of neem seeds at the highest concentration of 0.1 g/ml on the development of Asian Rust of soybean.

The MCI50 and MCI90 of the different extracts were determined with the *C. malayensis* strain tested. The lowest MIC values are obtained with the ethyl acetate and acetone extract, which justifies their efficacy and therefore their fungicidal and fungistatic potential. Doumbouya et al. [60] showed that indeed the low MIC values highlight the efficacy of an extract because they obtained a strong inhibition of the development of phytopathogenic fungi with extracts of *Ocimum graticinum*.

5. CONCLUSION

The general objective of this study was to evaluate *in vitro* the antifungal potential of *Annona muricata* seed extracts on Cercospora leaf spot disease caused by *Cercospora*

malayensis. Thus, the nature of the extraction solvent has a direct impact on the quantity and quality of *A. muricata* extracts for use as a fungicide. All the extracts tested inhibited the radial growth of *C. malayensis*. MIC was determined for those extracts that were found to be antifungal. The ethyl acetate extract was found to be the most effective against *C. malayensis.* The extracts were found to be potential antifungal to the *C. malayensis* strain and might be an alternative in the fight against fungal diseases of okra as their activity was comparable to that of the synthetic fungicide Monchamp 72 WP.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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