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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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’). 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 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].

\[
\text{Yield} \% = \frac{\text{Mass of extract} (g)}{\text{Mass of powder} (g)} \times 100
\]

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 Harborne [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 cm² 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 (3.33 g/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]:

\[ PI (%) = \frac{(Dc-Dx)}{Dc} \times 100 \]

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).

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

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

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

### 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 |

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 |
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 Omolar 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 Tsopmbeng 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. malayensis, 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 Cladosporium gloeosporioides. 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 Aspergillus 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

| Extrait   | 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.

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

5. CONCLUSION
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.

REFERENCES

1. Fondio L, Djidji HA, Kouame C, Traore D. Effet de la date de semis sur la production du Gombo (Abelmoschus spp.) dans le centre de la Côte d’Ivoire. Agronomie Africaine. 2003;15(1):13-34. DOI:10.4314/agaa.v15i1.1626
2. Hamon S, et Charrier A. Organisation évolutive du genre Abelmoschus (Gombo): co-adaptation et évolution de deux espèces de Gombo cultivées en Afrique de l’Ouest (A. esculentus et A. caillei). Ed. ORSTOM. Paris, France. 1987;191.
3. Charrier A, Les ressources génétiques du genre Abelmoschus Med. (Gombo). ORSTOM. 1983;81.
4. Akotag T, Hamon S, Koechlin J. The reproductive biology of Okra. 2. Self-fertilization kinetics in the cultivated okra (Abelmoschus esculentus), and consequences for breeding. Euphytica. 1990;53:49-55. DOI: 10.1007/BF00032032
5. FAO. Statistics databases; annual production of Okra in Africa. Available: http://www.fao.org/faostat/en (Accessed February 2021).
6. Doumbia M, Seif AA. Itinéraire technique pour le gombo en pays ACP. PIP. COLEACP-UGPIP. Bruxelles-Belgique. 2008;67.
7. Kumar S, Dagnoko S, Haougui A, Ratnadass A, Pasternak D, Kouame C. Okra (Abelmoschus spp.) in West and Central Africa: Potential and progress on its improvement. African Journal of Agricultural Research. 2010;5:3590-3598.
8. Eman S. H. Farrag. First record of Cercospora leaf spot disease on okra plants and its control in Egypt. Plant Pathology. 2011;10:175-180. DOI: 10.3923/ppo.2011.175.180
9. Hassan S, Dubey VK, Bhagat KP. Effect of insecticides and plant products against shoot and fruit borer of okra, Earias vitella (Fab.). Agric. Sci. Digest. 1998;18(2):120-122.
10. Jesus WC, Vale FX, Coelho RR, Haub Zambolin L, Costa LC, Bergamin FB. Effects of angular leaf spot and rust on yield loss of Phaseolus vulgaris L. Phytopathology. 2001;91:1045-1053.
11. Ambang Z, Ndongo B, Amayana D, Djilé B, Ngoh JP, Chewachong GM. Combined effect of host plant resistance and insecticide application on the development of cowpea viral diseases. Austr. J. Crp. Sci. 2009;3(3):167-172.
12. Pohe J, Agneron TA. L’huile des graines de neem, un fongicide alternatif à l’oxyde de cuivre dans la lutte contre la pourriture brune des cabosses de cacaoyer en Côte d’Ivoire. J. Appl. Biosci. 2013;62:4644-4652. DOI: 10.4314/jab.v62i0.86147
13. Ngassoum BM, Ngamo LS, Goudoum A. Protection post-récolte du maïs et ses insecticides peu rémanents: les huiles essentielles. In: Kapseu C., Nganhou J., Boudrant J. & Crouzet J. (eds). Séchage et technologie post-récolte. Cameroun. 2002:240-246.
14. Djeugap FJ, Fontem DA, Tapondjou AL. Efficacité in vitro et in vivo des extraits de plantes contre le mildiou (Phytophthora infestans) de la morelle noire. Int. J. Biol. Chem. Sci. 2011;5(6):2205-2213. DOI: 10.4314/ijbcs.v5i6.3
15. Makun HA, Anjorin ST, Adeniran LA, Onakpa MM, Muhammad HL, Obu OR. Toxic constituents of different provenances of Jatropha curcas and Ricinus communis seeds on Fusarium verticilliodes and other fungi.
Antimicrobial activities and phytochemical analysis of
Annona muricata
to determine its potential for novel medicines.

16. Abdel-Rahman T, Hussein AS, Beshir S, Hamed AR, Ali E, El-Tanany SS. Antimicrobial Activity of Terpenoids Extracted from Annona muricata Seeds and its Endophytic Aspergillus niger Strain SH3 Either Singly or in Combination. Open Access Maced. J. Med. Sci. 2019;7(19):3127-3131. DOI: 10.3889/oamjms.2019.793

17. Ambang Z, Ngo Dooh JP, Essono G, Bekolo N, Chewachong G, Asseng CC. Effect of Thevetia peruviana seeds extracts on in vitro growth of four strains of Phytophthora megakarya. Plant Omics Journal. 2010;3(3):70-76.

18. Mboussi SB, Ambang Z, Ndogo P, Ngo Dooh JP, Manga Essouma F. In vitro antifungal potential of aqueous seeds extracts of Azadirachta indica and Thevetia peruviana against Phytophthora megakarya in Cameroon. J. Appl. Life Sci. Int. 2016;4(4):1-12.

19. Essomé SC, Ngo Dooh JP, Heu A, Ndogo PA, Ngatsi ZP, Chewachong G, Ambang Z. Evaluation des activités antifongiques des extraits de graines de Thevetia peruviana contre Phytophthora colocasiae (Oomycètes) agent causal du mildiou du taro (Colocasia esculenta (L.) Schott. J. Appl. Biosci. 2020;151:15584-15597. DOI: 10.35759/JABs.151.7

20. Ngatsi ZP, Bekolo N, Yanga MNM, Tize Tiz, Azafack NS, Daouda K, Kuate TNW, Djéto-Lordon L. Effect of extracts from seeds of Thevetia peruviana (Pars.) K. Schum against cassava root scale Stictococcus vayssierei Richard (Hemiptera: Stictococcidae) in field. Int. J. Biosci. 2020;16(3):536-547. DOI: 10.12692/ijjb.16.3.536-547

21. Le Ven J. Contribution à l’étude du lien entre Annonaceae et parkinsonismes: identification et quantification d’acétogénines par déréplication; métabolisation de phase I et approche de la distribution de l’annonacine. Thèse de Doctorat, Université Paris-Sud 11. 2012;40-109.

22. Obugbuyiro JAO, Omotosho OE, Taiwo OS, Ononwụ FO, Banwo AS, Akintokun OA, Obaseki OS, Ogunleye OM. Antimicrobial activities and phytochemical properties of Annona muricata leaf. Coven J. Phys. Life Sci. 2017;5:40-49.
noire. Biosciences Proceedings. 2009;15: 85-92.

34. Tsopmbeng NG, Megtche CJP, Lienou JA, Yaouba A, Djeugap FJ, Fontem DA. Evaluation des activités antifongiques des extraits de plantes contre Phytophthora colocasiae, agent causal du mildiou du taro (C. esculentum (L) Schott). J. Appl. Biosci. 2014;81:7221-7232. DOI: 10.4314/jab.v8i11.2

35. Ondo AS. Caractérisation de quelques isolats de P. megakarya agent causal de lapourriture brune des cacaobois de cacaoyer (Theobroma cacao L). Mémoire de DEA, Université de Yaoundé I. 2005:58.

36. Nyassé S. Structure d’une population de phytophthora spp. Des cacaoyères camerounaises atteintes de pourriture brune. Mémoire de diplôme de recherche Universitaire ENSAT, Toulouse. 1992:43.

37. Vaz PDC. IMP Description of Fungi and Bacteria. 1987:92-916.

38. Hsieh WH, Goh TK. Cercospora and similar fungi from Taiwan. Maw Chang Book Compagny, Taiwan; 1990. Available:www.bccrc.firdi.org.tw/fungi/fungal

39. Singh G, Padavay RK, Narayanam CS, Padmhurmeri KP, Rao GP. Chemical and fungistatic investigation out the essential oil Citrus. Pers. Z. dentshe zeits haft für pflanzenfrankenen und flanzenschutz. 1993;100:69-74.

40. Pandey DK, Chandra H, Tripathi NN. Volatile fungitoxicity activity in higher plants special reference to that of Callistemon lanceolatus D.C. Phytopathology. 1982;105:175-182.

41. Kishore N, Mishra AK, Cham SYNN. Fungitoxicity of essential oil against dermatophytes. Mycoses. 1993;36:211-215.

42. Dohou N, Yamni K, Badoc A, Douira A. Activité antifongique d’extraits de Thymelaea lythroides sur trois champignons pathogènes du riz. Bull. Soc. Pharm. 2004;143:31-38.

43. Tsopmbeng GR, Lienou JA, Megagpche CJP, Fontem DA. Effect of pH and temperature levels on in vitro growth and sporulation of Phytophthora colocasiae, taro leaf blight pathogen. Int. J. Agro. Agri. Resch. 2014;4(4):202-206.

44. Muhammad Z, Sadia H, Komal R, Nasir R, Muhammad R, Zia-Ul-Haq M, Vincenzo DF. Antioxidant potential and oil composition of Callistemon viminalis leaves. Scientific World Journal. 2013;10: 11-55. DOI: 10.1155/2013/489071

45. Bruneton J. Phytochimie, Plantes médicinales. 3e édition Tec. et Doc., Lavoisier Paris. 1999;11-20.

46. Smallfield B. Introduction to growing herbs for essential oils, medicinal and culinary purposes. Crop & Food Research. 2001:45:1-4.

47. Valnet J. Aromatherapie: Traitement des maladies par les essences des plantes. 9e Ed. Maloine. 1980:510.

48. Omolara JO, Matthew OO, Abiola MA. Comparative phytochemistry and antioxidant activities of water and ethanol extract of Annona muricata leaf seed and fruit. Journal of Advances in Biological Research. 2016;10(4):230-235.

49. Naik, AV, Sellappan K. Physiochimical and phytochemical Analysis of different plant parts of Annona muricata L. (Annonaceae). Pharm Methods. 2019;10(2):70-78. DOI: 10.5530/phm.2019.2.13

50. Pamo TE, Tapondjou L, Temdonkeng F, Nzogang JF, Djoukeng J, Ngandou F, Kana JR. Effet des huiles essentielles des feuilles et des extrémités fleuries des Cypresse lussitanica sur la Tique (Rhipicephalus Lunulatus) à l’ouest Cameroun. Revue de l’Académie des Sciences du Cameroun. 2003;3(3):169-175.

51. Kone NAN, Ndongo B, Mountapmbeme MM, Manga EFR, Heu A, Mboussi SB, Ambang Z. Anti-fungal activities of Jatropha curcas seeds extracts against Cercospora malayensis causative agent of Sigatoka of Okra leaves. Inter. J. Sc. Resc. Methd. 2018;9(1):95-109.

52. Bautista BH, Lopez M, Bosquez ME, Wilson CL. Effect of extracts and plant extracts on growth of Colletotricum gloeosporioides, anthracnose and quality of papaya fruit. Crop Protection. 2003;1097-1092.

53. Reddy ISA, Fadipe VO, Akinremi OO, Bako SS. Variation in oil composition of Thevetia peruviana Juss “Yellow oleander” fruit seed. Journal of Applied Sciences and Environmental Management. 2002;8:61-66.

54. Gata-Gonçalves L, Nogueira JMF, Matos O, De Sousa BR. Photoactive extract from Thevetia peruviana with antifungal properties against Cladosporium cucumerinum. J. Photochem Photobiol B. 2003;70(1):51-54. DOI: 10.1016/s1011-1344(03)00024-1
55. Zirihi GN, Soro S, Kone D, Kouadio Y J. Activité antifongique de l'extrait naturel de *Combretum* sp. *in vitro* sur 3 espèces fongiques telluriques des cultures de tomates en Côte d'Ivoire. Rev. Ivoir. Sci. Technol. 2008;11:131-142.

56. Nchare SF. Evaluation du potentiel antifongique des extraits de graines de *Jatropha curcas* sur le développement *in vitro* des souches de *Phytophthora megakarya*. Mémoire de Master, Université de Yaoundé I. 2014;62.

57. Carlton R, Watermann R, Gray AI, Deans SG. The antifungal activity of leaf gland volatile oil of sweet gale (*Myrica gale*) Myricaceae. Chemaecology. 1992;3:55-59.

58. Soro S, Ouattara D, Zirihi DN, Kando C, N’guessan EK, Kone D. Kouadio JY, Ake S. Effet inhibiteur *in vitro* et *in vivo* de l'extrait de poudre et de l'huile essential de *Xylopia aethiopia* (dunal) A. Rich (Annonaceae) sur *Fusarium oxysporum* f. sp Radicis des cultures de tomates. European Journal of Scientific Research. 2010;30(2):279-288.

59. Ndogho PA, Ambang Z, Makanté P, Tchadjoko N, Gbapor G, Mvondo GD, Kone NN. Effect of aqueous extracts of neem seeds (*Azadirachta indica*) on the development of Asian Rust of soybean in the Center of Cameroon. International Journal of Environment Agriculture and Biotechnology. 2018;3(3):956-964.

60. Doumbouya M, Abo K, Lepengue HN, Camara B, Kanko K, Aidara D, Kone D. Activités comparées *in vitro* de deux fongicides de synthèse et de deux huiles essentielles sur les champignons telluriques des cultures maraîchères en Côte d’Ivoire. J. Appl. Biosci. 2012;50:3520-3532.

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