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Anti-microbial Effect of Cocoa Leaf Extracts on *Botryodiplodia theobromae*; Leading Causative Organism of Crown Rot Disease of Banana (*Musa acuminata*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

An investigation was carried to assess *in-vitro* management of crown rot disease on Banana using extracts of cocoa leaves. The causative organism (*Botryodiplodia theobromae)* was sub cultured and the concentration determined through microbial count. Different cocoa leaves extracts were prepared using two different solvents. A conventional fungicide known as Mancozeb was used as a positive control and the nutrient broth only served as negative controls to help in assessment of the

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antimicrobial effect of the cocoa leave extracts. Phenolic Methanol Extracts (PME), Crude Cocoa Leaves Aqueous Extracts (CCAE) and Mancozeb were employed to prepare aqueous solutions through serial dilution with varied concentration. The nutrient broth (100 µL of double strength), 100 µL each of the plant extract concentrations, and 20 µL of inoculums were dispensed in a sterile micro-titre plate containing 96 wells. Incubation of the micro-titre plate was carried out at a temperature of 37°C for 1 day (24 hours). Susceptibility or otherwise of the microorganism (*B. theobromae*) from four sample stations was analyzed with 20 µL of a 5% solution of *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* MTT. The presence of blue-black wells indicated the growth of *B. theobromae*. The experiment was conducted in triplicates for PME (refined), CCAE and Mancozeb. The minimum inhibition concentrations (MICs) of PME (refined) that inhibited the growth of *B. theobromae* collected from four different locations were 5mg/ml, 2.5 mg/ml, 10 mg/ml and 2.5 mg/ml respectively for *B. theobromae* $(B1)_{(Offinso)}$, *B. theobromae* $(B2)_{(Effiduase)}$, *B. theobromae* $(B4)_{(Kejetia)}$ and *B. theobromae* $(B5)_{(Ejisu)}$ which was lower compared to the MICs of CCAE which was 30 mg/ml for the four sample stations. Comparatively, the MIC of the PME (5 mg/ml, 2.5 m g/ml, 10 mg/ml and 2.5mg/ml) was almost equivalent to the MIC of the conventional fungicide (Mancozeb) (2.5 mg/ml) for all the four sample stations of *B. theobromae*.

Keywords: Antimicrobial activity; crown rot; phytochemical; polyphenol.

1. INTRODUCTION

The human population is fast increasing and the demand for healthy food is also increasing. However, numerous diseases continue to deteriorate food crops which leads to hunger. The increasing incidence of fungal diseases and their evolving resistance to fungicides have served as great motivation to enhance research efforts to develop environmentally safe and effective medicines as alternatives to control fungal diseases like crown rot disease. Plants continue to prove as potential sources of bioactive compounds or phytochemicals [1] such as phenolic which possess unique functions and ability to inhibit the growth and development of bacteria, fungi, insects and other microorganisms [2,3,4,1]. Many natural products and plants are potential sources for the development of biodegradable natural fungicide.

The crown rot disease; main postharvest disease [5,6], causes depreciation in banana quality during exportation which negatively affects trade [7,8,9]. The disease is of great concern to many including farmers, researchers, agriculturist and scientists. Effective control of the disease can be challenging and complicated [10,11,12]. Among the most prevalent organisms causing the crown rot in bananas identified at the four sample stations in this research are *Botryodiplodia theobromae, Colletotrichum spp., Colletotrichum germanium and Fusarium oxysporium* [9] Marin et al. 1996. *Botryodiplodia theobromae (B. theobromae)* also known as *Lasiodoplodia theobromae (L. theobromae)* is often related to the disease [13,14] and it's pathogenic [15,16].

Banana contains antioxidants with many health benefits. However, the use of chemicals such as Thiabendazole (TBZ), Mancozeb and Benomyl to reserve the fruit is detrimental to the health of consumers and harmful to the environment [16,17]. Therefore, research efforts and innovative methods are under exploitation towards finding sustainable solutions as alternatives to replace the current chemicals being used to preserve banana. The use of plant extracts in previous researches have exhibited some potent antibiotic and antifungal activities which will help safeguard consumer's health and protect the environment from chemical pollution.

The leaves of cocoa trees (*Theobroma cacao*) are one potential source of phenolic, phytochemicals and polyphenol compounds which are reported to have high antioxidant, anti-microbial and antifungal effects. Cocoa beans and related products may have an abundant deposit of polyphenols [18,19,20,21] making them very useful. The raw leaves have high polyphenols and antioxidants [22]. Polyphenolics inhibit microbial growth [23,24] and some researchers have also shown that polyphenolic can inhibit postharvest diseases in bananas [25,26].

By-products of cocoa processing are also potential sources of antioxidants. However, contrasting views still exist on examining the antimicrobial effects of cocoa and its polyphenolic extracts due to insufficient studies and discoveries. Some selected plant extracts have shown growth inhibition of many deadly organisms [27,28,1].

The main purpose of this research was to scrutinize the potency (antimicrobial or antifungal property) of cocoa leaves (CLs) extracts. Therefore, the focus of this study was to determine the minimum inhibitory concentrations (MICs) of the extracts on the growth of *Botryodiplodia theobromae*.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted at the Pathology Laboratories of Crop Research Institute (CRI) of the Center for Scientific and Industrial Research (CSIR) (CRI-CSIR) located at Fumesua in Kumasi and the Faculty of Pharmacy laboratories at Kwame Nkrumah University of Science and Technology (KNUST), Kumasi-Ghana.

2.2 Materials

The equipment and materials used for this experiment include needles, autoclave, flame, spatula, laminar flow, distilled water, forceps, Petri dishes, 200 ml conical flask, mass balance, microscope, potato dextrose agar (PDA), Micro-titre plates, 500 ml round bottom flask, analytical balance, volumetric flasks, convection oven dryer, incubator, distilled water, Improved Newbauer hemacytometer, *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* (MTT) etc. Sample organism is a purely cultured *Botryodiplodia theobromae.* The raw material for the extracts was young and fresh green cocoa leaves (CLs). The apparatus and reagents were accessed from the said laboratories.

2.3 Sources of B. theobromae

The microorganism (*B. theobromae*) used for this research was collected from four sample stations and stores of pure cultures previously isolated from crown rots of cavendish bananas from the Pathology Laboratories of CRI-CSIR.

2.4 Sample Labels

The organisms identified as *B. theobromae* $(B1)_{(Offinso)}$, *B. theobromae* $(B2)_{(Effiduase)}$, *B. theobromae* $(B3)_{(Kejetia)}$ and *B. theobromae* $(B4)_{(Ejisu)}$ were sampled from infected cavendish variety of bananas located at Offinso, Effiduase, Kejetia and Ejisu respectively within Kumasi in the Ashanti region, Ghana. They are also simply denoted as B1, B2, B3 and B4 about the above.

2.5 Spore Count

The concentration of the organism from the four sample stations was determined by spore count with a microscope using Improved Newbauer hemacytometer and a counter. The following formula was employed to compute the required sample concentrations;

$X=\frac{IC\times FV}{IV}$, where X= required concentration, IC= initial concentration, FV= final volume, IV= initial volume.

2.6 Medium Preparation

A homogeneous solution consisting of 19.5 g potato dextrose agar (PDA) and 500 ml distilled H_2O was prepared. The solution was autoclaved at a temperature of about 121°C and a pressure of 100 Pa for 0.25 hours (15 minutes) to help sterilize them (Johnston and Booth, 1983). After this, the medium (PDA) was cooled to surrounding air temperature whiles it remained liquid. It was then conveyed into plastic Petri dishes; 9 cm in diameter, autoclaved in a Lamina Flow Chamber until it solidified.

2.7 Sub Culturing

Stored pure single fungal cultures of *B. theobromae* were acquired from CRI-CSIR. Fungal isolates were conveyed onto the prepared PDA by cutting colonies of the fungi from the pure culture onto the PDA (regular sub culturing). It was then incubated for a 14-day period under a temperature of 28°C whiles the cultural, physical and morphological changes and characteristics of the fungus (*Botryodiplodia theobromae*) was examined under a microscope for confirmation purposes [29].

2.8 Collection and Preparation of CLs

The CLs were plucked from the cocoa plants (*Theobroma cacao)* as shown in Fig. 1. The CLs were well processed; steam-blanched at a temperature of 41°C for 2 minutes, to deactivate peroxidase enzymes present in the CLs. The CLs were then dried in a dryer (convection oven) at 45°C until residual moisture content recorded was 8% (w/w) dry basis [30].

2.8.1 Preparation of Crude Cocoa Leaves Aqueous Extract (CCAE)

Dried CLs were ground into fine powdered (<0.63 µ) form by using grinding machine. The

powdered CLs were sealed in plastic bags and stored in a desiccator, after which 500 g of powered CLs were subsequently extracted at ambient temperature with 4500 ml (4.5 liters) of sterile distilled water for 7 days. Within the 7 days, the extract was refrigerated for 5 nights. The filtrate (CCAE) obtained was condensed at 70°C in a water bath with a reddish-brown color. The CCAE is shown in Fig. 2.

2.8.2 Preparation of Phenolic Methanol Extract (PME)

Polyphenol extraction from the CLs with Methanol was conducted by employing Todd and Paul [31] method with some level of modification. 100 g of Dried CLs (in powdered form) was weighed and anhydrous methanol was used as the extracting reagent. A mixture (comprising of 100 g of powered CLs, methanol and sufficient H_2O) was prepared to keep the mass in liquid state. Sedimentation of the mixture was allowed to take place for about 1 hour 30 minutes, and the supernatant liquid (comprising of methanol) was evaporated from a rotary evaporator. The mixture was combined with 0.09 liters (90 ml) of hexane under agitation and two separate phases (water phase and water-insoluble hexane phase) were formed. The two phases were then separated from each other. The water phase was further treated with 0.03 liters (30 ml) of hexane. 10 g of NaCl to the water layer with H_3PO_4 to adjust the pH to about 3.5. The water (aqueous) phase was extracted twice using 150 ml $CH_3COOCH_2CH_3$ (ethyl acetate). A residue (dry solid catechin-rich fraction) was obtained after evaporation of the $CH_3COOCH_2CH_3$. A capped bottle was used to store the final $CH_3COOCH_2CH_3$ extract.

Fig. 1. Cocoa tree (*Theobroma cacao*)

Fig. 2. Phenolic Methanol Extract (PME) of young/fresh green cocoa leaves (CLs)

Fig. 3. Crude cocoa leaves aqueous extracts (CCAE)

2.9 Total Antioxidant Capacity

Employment of phosphomolybdate method by Prieto et al. (1999) with some level of modification was used to determine the total antioxidant capacity of the extracts. 3 ml volume of solution (made up of 0.6 M sulphuric acid, 28 nM Na_2HPO_4 and 4mM ammonium molybdate); serving as a positive control, was thoroughly mixed with 1 ml of each extract (0.03125-1 g/ml) in separate containers. Incubation of the mixtures was conducted at 95°C for about 1-hour 30 minutes and then cooled to ambient temperature condition. The absorbance (at 695 nm wavelength) was determined for 200 µl of the mixtures placed in micro-titre plate. Vitamin E (similarly processed) as a test drug was employed as a blank. Solutions of Vitamin E (in methanol) was employed to draw the standard curve. Expression of the total antioxidant value was in terms of Vitamin E (mg/g dry extract) equivalence.

2.10 Broth Micro-dilution Method

Condensed PME and CCAE were employed for the preparation of aqueous solutions through serial dilution at concentrations of 40, 20, 10, 5, 2.5 and 1.25 (mg/ml) and 60, 50, 40, 30, 20, 10, 5 and 2.5 (mg/ml) respectively. Since the respective extracts' concentrations were mixed with an equal volume of nutrient broth (of double strength), eventual concentrations of extracts in-well were between 30 mg/ml to 0.3125 mg/ml. A nutrient broth (100 µL of double strength), 100µL each of the plant extract concentrations, and 20 µL of inoculums were dispensed in each well of a sterile micro-titre plate which contained 96 wells. Incubation of the micro-titre plate was carried out at a temperature of 37°C for 1 day (24 hours). Susceptibility or otherwise of the microorganism (*B. theobromae*) to the extracts from the four sample stations was analyzed with 20 µL of a 5% solution of *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* MTT. The nutrient broth used in the experiment only served as negative controls and a conventional fungicide (Mancozeb) in the concentrations of 40, 20, 10, 5, 2.5 and 1.25 (mg/ml) was used as positive controls. The experiment was carried out in triplicates with the PME, CCAE and Mancozeb.

2.11 pH test of Extracts

Prepared PME and CCAE were respectively mixed with distilled water at a ratio of 2:5. pH meter was used to record their respective pH.

3. RESULTS

3.1 Identification and Characterization of Fungi Isolates

Collected samples (*B. theobromae* $(B1)_{(Offinso)}$, *B. theobromae* $(B2)_{(Effiduase)}$, *B. theobromae* $(B3)_{(Kejetia)}$ and *B. theobromae* $(B4)_{(Ejisu)}$) were first carefully examined to ascertain the presence of the fungi (*B. theobromae*) by physical observation with the aid of a microscope. The organisms or fungi (from the four sample stations) exhibited greyish brown culture which is characteristic of fungi worldwide. The fungi also formed "pycnidia" which had black projection in nature. The fungal spores were oval, with matured spores appearing dark with a single septum while young and immature spores were colourless and unicellular. The preliminary growth looked snow-white and appeared greyish black after maturity. Upon comparing the physical, cultural and morphological changes and the characterization of *B. theobromae* isolated from the bananas with shape, colour, size and structure as described by Mathur and Kongsdal (2003), it was concluded that the fungi collected was indeed *Botryodiplodia theobromae*.

3.2 Spore Count

Figure 4 shows the concentrations of sample *B. theobromae* $(B1)_{(Offinso)}$, *B. theobromae* $(B2)_{(Effiduase)}$, *B. theobromae* $(B3)_{(Kejetia)}$ and *B. theobromae* $(B4)_{(Ejisu)}$ recorded following the spore count as 2.2×10^6, 3.9×10^6, 3.3×10^6, 4.6×10^6 (s/ml) respectively.

Table 1. pH test on Crude Cocoa Leave Aqueous Extract (CCAE) and Phenol-Methanol Extract (PMP) of young cocoa leaves (CL)

Extract	Acidity
Phenol-Methanol Extract (PME)	+
Crude CL-Aqueous Extract (CCAE)	+

+ = *Acidic*

3.3 pH of Extracts

After dilution of PME and CCAE in the ratio 2:5 with distilled water and pH determination, the 2 extracts turned the litmus paper from blue to red indicating that both extracts were acidic in nature (Table 1).

3.4 Assessment of Minimum Inhibitory Concentration (MIC)

Susceptibility or otherwise of *B. theobromae* (from the four sample stations) to the extracts were analyzed using 20 µL with a 5% solution of MTT (*3-(4,5-dimethylthiazol-2-yl)-2,5-dipheny-ltetrazolium bromide*). The presence of blue-black wells indicated the growth of *B. theobromae* in the presence of the extracts.

3.4.1 MIC of PME

Table 2 indicates the antimicrobial activity of PME on *B. theobromae* after 24 hours incubation at a temperature of 37°C. *B. Theobromae* $(B1)_{(Offinso)}$ recorded MIC of 5 mg/ml. *B. theobromae* $(B2)_{(Effiduase)}$ recorded a lower MIC of 2.5 mg/ml. *B. theobromae* $(B1)_{(Kejetia)}$ also recorded MIC of 10 mg/ml and finally *B. theobromae* $(B1)_{(Ejisu)}$ recorded MIC of 2.5 mg/ml. At concentration "G" only the microorganisms (*B. theobromae*) and broth were applied (no extract applied). At "G" the microorganisms were active after 24 hours incubation period. In concentration "H" only broth was applied and no activity of microorganisms after incubation for a day, which indicates that the broth used had no microbial contamination.

3.4.2 Crude Cocoa Leaves Aqueous Extract (CCAE)

Table 3 demonstrates the antimicrobial property of CCAE after 1 day (24 hours) incubation at a temperature of 37°C on *Botryodiplodia theobromae*. *B. Theobromae* $(B1)_{(Offinso)}$, *B. theobromae* $(B2)_{(Effiduase)}$, *B. theobromae* $(B3)_{(Kejetia)}$ and *B. theobromae* $(B4)_{(Ejisu)}$ all recorded the same MICs of 30 mg/ml with concentrations varying from 60 mg/ml to 2.5 mg/ml.

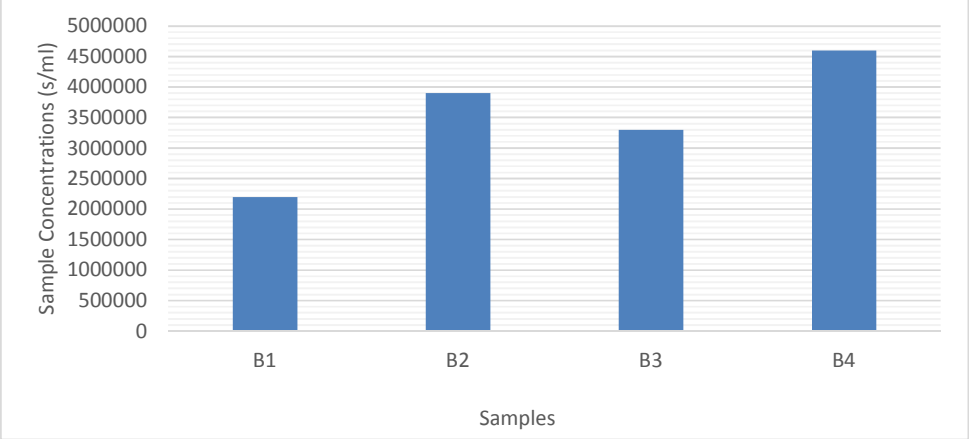

Fig. 4. Spore count of samples; *B. theobromae* $(B1)_{(Offinso)}$, *B. theobromae* $(B2)_{(Effiduase)}$, *B. theobromae* $(B3)_{(Kejetia)}$ and *B. theobromae* $(B4)_{(Ejisu)}$

Table 2. Antimicrobial test of Phenolic Methanol Extracts (PME) of young leaves of cocoa tree (*Theobroma cacao)* on *Botryodiplodia theobromae*

	***Botryodiplodia theobromae* sampled from different locations**				
Concentrations of extract (mg/ml)	**mg/ml**	***B. theobromae* $(B1)_{(Offinso)}$**	***B. theobromae* $(B2)_{(Effiduase)}$**	***B. theobromae* $(B3)_{(Kejetia)}$**	***B. theobromae* $(B4)_{(Ejisu)}$**
	40	-	-	-	-
	20	-	-	-	-
	10	-	-	-	-
	5	-	-	+	-
	2.5	+	-	+	-
	1.25	+	+	+	+
	G	+	+	+	+
	H	-	-	-	-

+ = no growth inhibition of B. theobromae
- = growth inhibition of B. theobromae

Table 3. CCAE antimicrobial property test on *Botryodiplodia theobromae*

	Botryodiplodia theobromae from different locations				
Concentrations of extract (mg/ml)	mg/ml	*B. theobromae* $(B1)_{(Offinso)}$	*B. theobromae* $(B2)_{(Effiduase)}$	*B. theobromae* $(B3)_{(Kejetia)}$	*B. theobromae* $(B4)_{(Ejisu)}$
	60	-	-	-	-
	50	-	-	-	-
	40	-	-	-	-
	30	-	-	-	-
	20	+	+	+	+
	10	+	+	+	+
	5	+	+	+	+
	2.5	+	+	+	+

+ = no growth inhibition of B. theobromae
- = growth inhibition of B. theobromae

3.4.3 Conventional mancozeb fungicide

A test was conducted to compare the antimicrobial properties of the extracts (PME and CCAE) clearly with Mancozeb (a conventional fungicide widely used by farmers and industries to control the crown rot disease in bananas). The objective of this test and analysis was to help assess and appreciate the potency of the extracts. From Table 4, *B. Theobromae* $(B1)_{(Offinso)}$, *B. theobromae* $(B2)_{(Effiduase)}$, *B. theobromae* $(B3)_{(Kejetia)}$ and *B. theobromae* $(B4)_{(Ejisu)}$ all recorded the same MIC of 2.5 mg/ml with concentrations varying from 40 mg/ml to 1.25 mg/ml. Only broth and Mancozeb were applied at concentration "H" and no microbial activity was recorded which indicates that the Mancozeb had no microbial contamination.

3.4.4 MICs of PME, CCAE and Mancozeb

From Fig. 5, the following conclusion was drawn. The average MICs of *B. theobromae* $(B1)_{(Offinso)}$ were 5 mg/ml, 2.5 mg/ml and 30 mg/ml for refined PME, Mancozeb, and CCAE respectively. The average MICs for *B. theobromae* $(B2)_{(Effiduase)}$ were 2.5 mg/ml, 2.5 mg/ml and 30mg/ml for PME, Mancozeb, and CCAE respectively. The average MICs for B. theobromae $(B3)_{(Kejetia)}$ were 10mg/ml, 2.5mg/ml and 30 mg/ml for PME, Mancozeb, and CCAE respectively. The average MICs for B. theobromae $(B4)_{(Ejisu)}$ were 2.5mg/ml, 2.5 mg/ml and 30mg/ml for PME, Mancozeb, and CCAE respectively.

4. DISCUSSION

4.1 Anti-fungal Properties of Selected Plants

The antimicrobial or the antifungal properties of PME, CCAE and Mancozeb were evaluated. *B. theobromae* was sampled from four different locations (Offinso, Effiduase, Kejetia and Ejisu) and treated with the extracts at varying concentrations, and their MICs were determined and evaluated.

The outcome of the research shows that the CLs extracts (PME and CCAE) were able to successfully inhibit the growth of microorganism (*B. theobromae*). The MICs of CLs extracts (PME and CCAE) demonstrate their ability to inhibit the growth of fungal microbes. The CCAE and PME of CLs were tested on the disease's (crown rot of banana) causative organism (*Botryodiplodia theobromae*) which indicated that they possess antifungal or antimicrobial properties.

Many research outcomes have confirmed antioxidant, antibiotic and antimicrobial activities of many selected plants which contain bioactive or phytochemical compounds [4,32,33,27,28, 34,35,1]. Research has shown that *O. gratissimum* and *A. melegueta* have proved to possess antifungal effects against spore germination and mycelia growth of many rot causing microorganisms. Research conducted by Amadioha [36] demonstrated that *O. gratissimum* leaf extracts were capable of controlling spore germination and mycelia growth of *Rhizopus oryzae* just as cocoa leaves extracts has proven to have shown its potency in controlling *B. theobromae*. Koomson et al., [1] also confirmed the composition of some phytochemicals in ethanol extracts of mature and young five *Solanum torvum* fruits. Phytochemicals such as saponins, phenols, alkaloids, tannins, glycosides [32,27,34] possess antimicrobial and antibiotic properties on many disease-causing organisms [1]. These research outcomes have wide useful applications in many fields including food processing, pharmaceuticals, agriculture etc. From the results, the conclusion drawn was that when the MIC of Mancozeb (< 2.5 mg/ml), PME

(< 2.5 mg/ml) and CCAE (< 30 mg/ml), their antifungal activities on *Botryodiplodia theobromae* becomes ineffective. However, when the MIC of Mancozeb (≥ 2.5 mg/ml), PME (≥ 2.5 mg/ml) and CCAE (≥ 30 mg/ml), their antifungal effects on *Botryodiplodia theobromae* becomes very effective. Besides, the application of PME in all the four sample stations may require a concentration of about 10 mg/ml. The cocoa leave extracts (PME and CCAE) can be said to contain some bioactive or phytochemical compounds which inhibited the growth of *Botryodiplodia theobromae.*

4.2 Evaluation of MICs of CLs extracts and Mancozeb

4.2.1 MIC of CCAE

From Fig. 5, it is evident that the CCAE required the highest concentration (MIC of 30 mg/ml) to inhibit the fungi (*B. theobromae*) growth compared to PME and Mancozeb (MIC 2.5 mg/ml). However, there was no variation in the concentration of CCAE needed to inhibit the fungi, considering the various locations the organism was collected. This denotes that the potency of CCAE (MIC ≥ 30 mg/ml) was the same irrespective of the locations the fungus was collected from.

4.2.2 MIC of PME

Application of PME on *B. theobromae* from the four localities ranged from 10 mg/ml to 2.5 mg/ml. This demonstrates some level of variation in the minimum concentrations that could prevent the fungi from growing and it connotes that the active ingredients present in the extract (PME) vary. Another reason may be that *B. theobromae* possess some level of resistance to PME which might also depend on *B. theobromae* morphological changes or adaptation to environmental conditions where they inhabit.

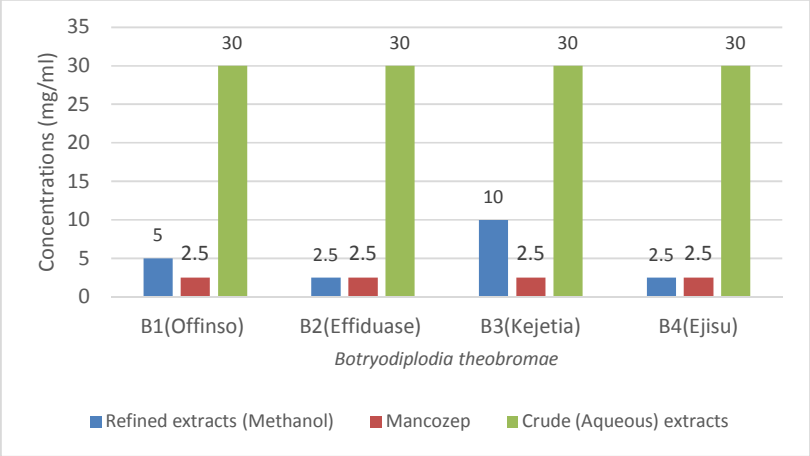

Fig. 5. Comparison between the minimum inhibitory concentrations (MICs) of PME, CCAE and Mancozeb

Table 4. Antifungal property of Mancozeb (conventional chemical fungicide) on *Botryodiplodia theobromae*

	***Botryodiplodia theobromae* from different locations**				
Concentrations of Mancozeb (mg/ml)	**Conc.mg/ml**	***B. theobromae* (B1)$_{(Offinso)}$**	***B. theobromae* (B2)$_{(Effiduase)}$**	***B. theobromae* (B3)$_{(Kejetia)}$**	***B. theobromae* (B4)$_{(Ejisu)}$**
	40	-	-	-	-
	20	-	-	-	-
	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
	1.25	+	+	+	+
	G	+	+	+	+
	H	-	-	-	-

+ = no growth inhibition of B. theobromae; - = growth inhibition of B. theobromae

The phytochemical analysis conducted on *Z. officinale, C. odorata, O. gratissimum and C. citratus* showed positive for most phytochemicals tested, with few exceptions [36,37,38,39]. The demonstrated antifungal potential of most plants is due to the presence of biologically active contents. The quantity of bioactive compounds or photochemical constituents in the extracts of most selected plants are influenced by many factors such as the age of the plant, extraction solvent(s) used extraction method(s) employed and time (season or climatic conditions) of harvested plant materials [28,1]. The medicinal, pharmacological and antimicrobial values of plants lies in their component phytochemicals [28,1]. Similarly, the age of the plant, extracting solvent, method of extraction of phytochemicals (polyphenols) and time of harvesting of the cocoa leaves had an influence on its active ingredients (bioactive contents). This may be one of the reasons for the varying MICs recorded for PME. The MIC indicates the minimum concentration of the extract necessary to prevent the growth and development of *B. theobromae.*

4.2.3 MIC of Mancozeb

Observations from the test conducted on Mancozeb demonstrated inhibitory effects on the mycelia growth of *B. theobromae*. The MICs of Mancozeb (2.5 mg/ml) was the same for all the treatments and replicates. This result confirmed the potency and the MIC of Mancozeb as recommended by its chemical manufacturer. The results obtained confirmed the antifungal activity of the extracts (PME and CCAE) but the methods of extraction showed some variations in the effectiveness (in terms of MICs) of PME and CCAE in controlling the causative microorganism (*B. theobromae*) of banana crown rot.

The average MIC of PME (5 mg/ml) was lower compared to all the MICs of CCAE (30 mg/ml) at the four sample stations. Comparatively, the lowest MIC of PME (2.5 mg/ml) is the same as the MIC of conventional fungicide (Mancozeb) (2.5 mg/ml). The extracts are great (natural, safe and biodegradable) potential fungicides to replace current chemical fungicides like Mancozeb which poses many health and environmental problems.

5. CONCLUSION

The outcomes of this experiment have shown that the crown rot of banana can be controlled by using extracts (PME and CCAE) of CLs. Both crude and refined phenolic methanol extracts of CLs showed antifungal activity against pathogenic *Botryodiplodia theobromae.* The MIC of refined PME and CCAE are 2.5 mg/ml and 30 mg/ml respectively. A control experiment was conducted on *B. theobromae* with Mancozeb (a conventional chemical fungicide). The MIC of Mancozeb is 2.5mg/ml. Comparing the MICs of PME and CCAE to that of Mancozeb, the conclusion drawn was that the extracts demonstrate a strong antimicrobial or antifungal property. PME and CCAE inhibited the growth of *B. theobromae;* one of the leading causative microorganisms of banana crown rot which is prevalent in Ghana. The use of these extracts will reduce over dependence on the current commercial fungicides or synthetic chemicals which are not only expensive to purchase for most farmers but also causes potential danger to the health of farmers, banana consumers and handlers as well as the environment at large. With a great rise in demand for organic fungicide due to residual contamination of food by inorganic chemical fungicides as well as issues relating to affordability, the PME of CLs can be used as a substitute or alternative to Mancozeb and other inorganic (chemical) fungicides to control the plant pathogen (*B. theobromae*) and other microbes. The use of plant extracts is an environmentally friendly approach and an effective alternative to toxic chemical fungicides. Employment of plant extracts such as PME and CCAE will also promote affordability and this will boost banana production and exportation. It will provide value for money and enhance economic activities. Further extensive researches are recommended to test the antimicrobial and antioxidant or antibiotic properties of different parts and products of *Theobroma cacao* and other plants within the same family (*Malvaceae*) for wider useful applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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