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***In vitro* efficacy of some plant aqueous extracts against two species of *Lasiodiplodia* associated to mango decline in Burkina Faso**

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ABSTRACT

Mango decline is a serious disease in production areas in Burkina Faso. The aim of this study was to contribute to the management of the disease through the use of plant aqueous extracts. Antifungal activities of *Azadirachta indica*, *Calotropis procera*, *Gmelina arborea*, *Jatropha curcas*, *Eucalyptus camaldulensis* and the synthetic fungicide (Mancozeb) were tested against *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* associated to mango decline in Burkina Faso. Three different concentrations of leaf extracts which 25%, 50%, 75% and 500 ppm of Mancozeb were tested for their antifungal activity *in vitro*. The results showed that leaf extracts have an inhibitor effect on the growth of the two *Lasiodiplodia* species. The aqueous extract of *G. arborea* was the most effective with average inhibition rates of *L. theobromae* of 42.62%, 73.84% and 74.23% respectively with the concentrations of 25 g/l, 50 g/l and 75 g/l. The aqueous extract of *A. indica* against *L. pseudotheobromae* showed maximum percentage inhibition with 50 g/l of 63.10% and with 75 g/l of 72.02%. Mancozeb completely inhibits the mycelial growth of both species of fungi. Ours findings showed that aqueous extracts from plants could be tried for the eco-friendly management of mango decline pathogens.

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Keywords: Antifungal, plants extract, *Lasiodiplodia* spp., mango decline, Burkina Faso.

INTRODUCTION

Mango (*Mangifera indica* L.) is a tree species producing delicious dessert fruits in the Anacardiaceae family. Mango stands at the

first position in fruit production in Burkina Faso and is therefore a crop of considerable economic importance for the country with a high potential for international (APROMAB,

2018). The mango sector employs many producers and many other actors such as consumers, processors, exporters, traders and transporters who live from production and its by-products. In 2018, fresh mango production has increased from around 90 000 tons in 2017 to 200 000 tons (APROMAB, 2018). In terms of resources, marketing of fresh and dried mangoes generated more than 21 617 643.11 USD. *M. indica* is susceptible to a number of diseases at all stages of its development (Alemu et al., 2014). Fungal pathogens are the most common agents causing diseases on mango, including dieback, a form of progressive death (Ismail et al., 2012). The symptoms of dieback are commonly associated with drying and withering of twigs from top to downwards, followed by discoloration, drying and eventual dropping of leaves (Saeed et al., 2017 ; Dianda et al., 2018; Dianda, 2019). Typically, a complete wilting and death of the affected mango trees may occur within weeks or few months after first symptoms occurred (Saeed et al., 2017). Dieback is considered to be the most destructive disease of the mango tree, leading to significant yield loss and low fruit quality (Saeed et al., 2017). Studies have identified fungi of several *Botryosphaeriaceae* species, such as *L. theobromae* (Pat.) Griffon and Maubl. (Sutton, 1980) or *L. pseudotheobromae* as the causal agents of mango dieback disease in different areas of the world, including Burkina Faso (Khanzada et al., 2004; de Oliveira Costa et al., 2010; Ismail et al., 2012; Ablormeti, 2016; Rodríguez-Gálvez et al., 2017; Saeed et al., 2017; Dianda, 2019). *Lasiodiplodia* species have been associated with several others disease symptoms on mango plants including fruit rot, stem-end rot, panicle brown rot, canker (Sakalidis et al., 2011; Ismail et al., 2012).

The unbalanced use of fertilizer, intercropping in the tree grooves, zero pruning and certain abiotic constraints such as drought,

high temperatures, sunshine, water stress, salinity and nutritional deficiencies can contribute to the development and progression of the decline caused by *L. theobromae* (Naqvi and Perven, 2015). The common strategy for controlling mango decline is using chemical fungicides with high efficiency. Such fungicide including Carbendazim, Fuguran, Mancozeb are generally used to control fungal pathogens by spraying copiously the infected plants (Ablormeti, 2016; Tedihou et al., 2017). However, due to growing concerns about the potential risk that fungicides pose for human health, environmental contamination, and the development of fungicide resistance by pathogens (El Ghaouth et al., 2003; Lema et al., 2014) a control strategy based on antagonistic microorganisms, or biocontrol, has become an attractive alternative approach. Plants and their derivatives have been extensively studied for the control of pathogenic fungi. Indeed, the extracts of acalypha (*Acalypha hispida*), siam weed (*Chromolaena odorata*), aidan (*Tetrapleura tetraptera*), and neem (*A. indica*) were reported to significantly inhibit *L. theobromae* isolates of cashew inflorescence blight in Nigeria (Dele and Abiodun, 2015). In another study, the extracts of *J. curcas* had inhibitory effects against this fungal, pathogenic on *Rhizophora racemosa* (Ukoima et al., 2013). In Pakistan, *in vitro* evaluation of the effectiveness of four plants extracts species PEs) against the causal agent of mango dieback revealed that safeda (*E. camaldulensis*) and Neem extracts were the most effective while garlic (*Allium sativum*) and onion (*Allium cepa*) extracts were comparatively and statistically less effective in inhibiting the vegetative growth of the fungus causal agent of quick decline of mango (Sahi et al., 2012). Moreover, *D. stramonium* and *E. camaldulensis* extracts reduced the growth of *L. theobromae* causing stem end rot of mango

in Pakistan more efficiently than some Aloe-
vera extract (Ullah et al., 2017).

Given the threat of decline on mango
production and the harmful effects linked to the
use of pesticides in Burkina Faso, this study
aimed at assessing the antifungal activity of
extracts of certain plant species against *L.*
theobromae and *L. pseudotheobromae* isolated
from diseased mango trees in Burkina Faso.

MATERIALS AND METHODS

Source of plant materials

The plants used in this study were
collected at Farako-Bâ site located at 10 km
from Bobo Dioulasso. They were selected
based on their medicinal properties reported by
several authors (Sahi et al., 2012; Dele and
Abiodun, 2015) and availability from farm
lands within Farako-Bâ near Bobo-Dioulasso.
These plants include *A. indica*, *C. procera*, *J.*
curcas, *G. arborae* and *E. camalulensis*
(Figure 1). Fresh leaves were manually plucked
and immediately transported in black
polyethylene bags to the laboratory for the
aqueous extraction.

Fungal isolates

One strain of *L. theobromae* (K11B)
and one strain of *L. pseudotheobromae* (H6B)
stored in the mycology laboratory of Institute
for the Environment and Agricultural Research
(INERA) of Bobo-Dioulasso were used for this
study. They were previously isolated from
mango trees with dieback symptoms. *L.*
theobromae and *L. pseudotheobromae* are
originated from Koloko
(N11°0571.14'W001°078.39') and Peni
(N11°046.080°W004°366.6') in Hauts Bassins
region, one of major area of mango production
in Burkina Faso. The fungal isolates were
identified based on DNA sequences of the
internal transcribed spacer region (ITS),
translation elongation factor (Tef1-alpha) and
beta-tubulin (Bt) (White et al., 1990; Alves et

al., 2008; Donaldson and GLass, 1995).
Pathogenicity of these strains was confirmed
by the detached leaves method described by
Ismail, (2011) and Munirah et al. (2017).

Preparation of aqueous extracts

The fresh leaves were first washed with
running tap water, then sterilized with 1%
sodium hypochloride and finally rinsed with
sterile distilled water.

They were then dried at laboratory
temperature on blotting paper for two weeks.
The dried leaves were crushed with a pestle in
a sterilized mortar and suspended in sterile
water at a ration 1:1 (w / v) according to the
method described by Rahee et al. (2018). The
aqueous extracts were filtered through two (02)
folds muslin clothes and centrifuged at 4000
rpm for 15 mn. The supernatant collected,
considered to be 100% concentration was
stored at 4 °C until subsequent use.

In vitro antifungal activity assay

Three different concentrations (C1:
25%; C2: 50% and C3: 75%) were prepared
from the stock solution (C0: 100%) to test their
ability to inhibit the mycelial growth of the two
species of *Lasiodiplodia*. Each concentration
(v) of aqueous extract was inoculated into PDA
medium maintained in a 55 °C water bath. The
mixture was then poured into Petri dishes. PDA
medium without aqueous extract and PDA
inoculated with Mancozeb at 500 ppm were
used as negative and positive controls,
respectively. A 5 mm mycelial disc was cut
from the actively growing regions (peripheral
mycelium) of 7-days old culture of the two
species of *Lasiodiplodia* and deposited on the
medium inoculated with aqueous plant
extracts. After inoculation, the plates were
incubated in the inverted position at 28 ± 2 °C.
The efficacy of aqueous extract was assessed
when the fungal completely cover the medium
used as a negative test. Each treatment was

replicated in ten plates. The rate of inhibition (PI) area induced by each treatment on the both fungal was calculated follow Ehiobu and Ogu, (2018) with formula :

$$\text{Mycelial growth inhibition} = \frac{(\text{Diameter of control} - \text{diameter of treatment})}{\text{diameter of control}} \times 100$$

Statistical analysis

The Excel 2013 spreadsheet was used for data entry and organization. Experimental data were analysed using R software version 3.6.2. The extracts of the five plant species were compared to each other in pairs and to the controls using the Wilcox test. This test made it possible to verify if there are significant differences between the different treatments in the inhibition of mycelial growth of the two species of *Lasiodiplodia* at the probability level of $P < 0.05$.



Figure 1: Photographs of the five selected medicinal plants in the field when leaves were collected. (A: *A. indica*; B: *G. arborea*; C: *C. procera*; D: *E. camaldulensis*; E: *J. curcas*).

RESULTS

The two *Lasiodiplodia* strains recorded a maximum mycelial growth in all negative control plates 72 hours after incubation (hai). However, their growth varied according to the extracts from five plant species used and the three concentrations tested (Figure 2 and Table 1).

Effects of plant extracts on the mycelial growth of the *L. theobromae* and *L. pseudotheobromae*.

Fungal growth observation at 72 h after incubation showed that the aqueous extracts of *A. indica*, *J. curcas*, *C. procera*, *G. arborea* and *E. camelendis* had antifungal properties against the two *Lasiodiplodia* species tested at for all the levels of concentrations tested 72h after incubation (Table 1). Highest growth inhibition was recorded at the concentration of 75% for all extracts. Except for *Eucalyptus*, extracts from the other four plant species generated mycelial growth inhibition rates greater than 50%. At the dose 75%, *G. arborea* aqueous extract inhibits the growth of *L. theobromae* with mean rate of 74.23%. On the other hand, *C. procera* recorded 72.20% of inhibition rate on the mycelial growth of *L. pseudotheobromae*. At 50% concentration, aqueous extracts of *G. arborea* and *A. indica* induced respectively inhibition rates of 73.87% and 63.10% of *L. theobromae* and *L. pseudotheobromae*. The lowest inhibition rates of the both fungal species were observed with

the all aqueous plants extracts at 25% concentration. Mancozeb used as a positive control inhibits totally the mycelium growth of the two species of *Lasiodiplodia* screened.

Efficacy of extracts from five plant species on *L. theobromae*

Table 2 shows the p-values obtained from the two-by-two comparisons of the plant extracts on *L. theobromae* using Wilcoxon-test. At the concentration of 75%, a significant difference was observed between the inhibition rates of the five plant extracts and the negative control. Among the aqueous extracts, that of *G. arborea* was found to be the most effective against *L. theobromae*. It showed significant differences with those of four other plant species. Statistically, the effect was not significantly different ($P < 0.05$) between the extracts of *A. indica* and *C. procera*.

Efficacy of extracts from five plant species on *L. pseudotheobromae*

Results of Table 3 and Figure 3 showed significant difference in per cent inhibition of *L. pseudotheobromae* by extracts of five plants species with the control. Among extracts of the five plants species tested, maximum percentage inhibition was recorded with *C. procera*. It didn't show significant difference with *A. indica* and *J. curcas*. Moreover, there is no significant difference between *J. curcas* and *G. arborea*.

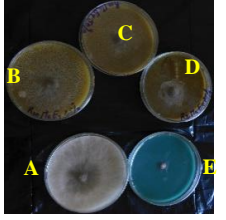
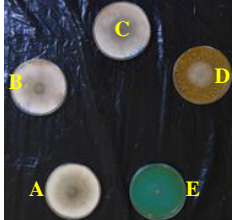
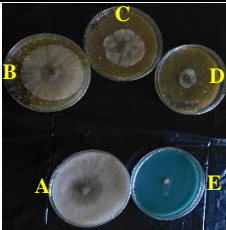
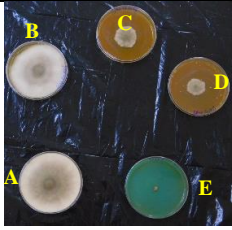

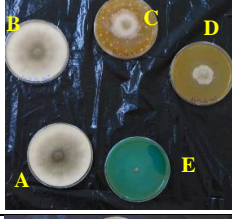
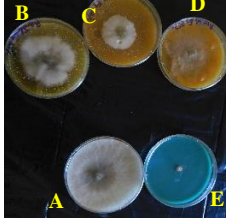

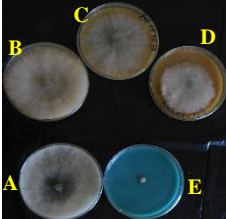
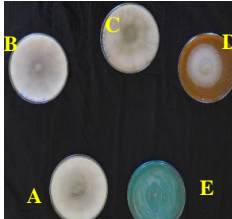
Plant extracts	Fungal	
	<i>L. theobromae</i>	<i>L. pseudotheobromae</i>
<i>G. arborea</i>		
<i>A. indica</i>		
<i>C. procera</i>		
<i>J. curcas</i>		
<i>E. camalendis</i>		

Figure 2: Effect of extracts of five plant species and Mancozeb (synthesis fungicide) on the mycelial growth of the two *Lasiodiplodia* species 72 hours after incubation (dose used: **A:** 0 (control); **B:** 25%; **C:** 50%; **D:** 75%; **E:** 500 ppm (Mancozeb)).

Table 1: Inhibition rates (%) of aqueous plant extracts on *L. theobromae* and *L. pseudotheobromae* after 72 h incubation.

Treatments	Concentrations	Inhibition over control (%)	
		<i>Lasiodiplodia theobromae</i>	<i>Lasiodiplodia pseudotheobromae</i>
<i>C. procera</i>	C1	59.94 ± 2.35	72.20 ± 5.20
	C2	38.87 ± 6.18	43.10 ± 6.26
	C3	32.62 ± 5.73	1.19 ± 1.25
<i>G. arborea</i>	C1	74.23 ± 6.74	68.15 ± 2.85
	C2	73.87 ± 7.20	11.13 ± 2.66
	C3	42.62 ± 5.45	6.19 ± 1.72
<i>J. curcas</i>	C1	54.05 ± 4.60	64.40 ± 8.78
	C2	42.32 ± 5.19	56.49 ± 8.69
	C3	31.43 ± 4.67	13.92 ± 2.79
<i>A. indica</i>	C1	60.12 ± 4.98	72.02 ± 5.01
	C2	42.74 ± 3.50	63.10 ± 5.62
	C3	33.99±9.35	1.07 ± 2.28
<i>E. camaldulensis</i>	C1	37.26 ± 4.44	35.12 ± 7.26
	C2	19.35 ± 3.54	4.82 ± 1.49
	C3	4.88 ± 3.14	1.01 ± 0.38
Idefix	500ppm	100.00 ± 0.00	100.00 ± 0.00
Control		0.00 ± 0.00	0.00 ± 0.00

Legend (C1= 75%, C2=50%, C3=25%).

Table 2: Comparisons between of extracts efficacy of five species plants and the control on *L. theobromae* using Wilcox-test.

	<i>A. indica</i>	<i>E. camaldulensis</i>	<i>C. procera</i>	<i>G. arborea</i>	<i>J. curcas</i>	Control
<i>A. indica</i>	–	0.0001817	0.4863	0.001137	0.008979	6.34e-05
<i>E. camaldensis</i>	0.0001817	–	0.0001817	0.0001817	0.0001806	6.386e-05
<i>C. procera</i>	0.4863	0.0001817	–	0.0002087	0.004495	6.34e-05
<i>G. arborea</i>	0.001137	0.0001817	0.0002087	–	0.0002422	6.34e-05
<i>J. curcas</i>	0.008979	0.0001806	0.004495	0.0002422	–	6.294e-05
Control	6.34e-05	6.386e-05	6.34e-05	6.34e-05	6.294e-05	–

P-values inferior at 0.05 in a column show a significantly different between two treatments (plant extracts) or one with the control.

Table 3: Comparison of extracts efficacy of five species plants on *L. pseudotheobromae* using Wilcox-test.

	<i>A. indica</i>	<i>E. camaldensis</i>	<i>C. procera</i>	<i>G. arborea</i>	<i>J. curcas</i>	control
<i>A. indica</i>	–	0.0001817	0.8198	0.02825	0.09593	6.386e-05
<i>E. camaldensis</i>	0.0001817	–	0.0001776	0.0001806	0.0001817	6.203e-05
<i>C. procera</i>	0.8198	0.0001776	–	0.02065	0.1197	6.203e-05
<i>G. arborea</i>	0.02825	0.0001806	0.02065	–	0.2406	6.34e-05
<i>J. curcas</i>	0.09593	0.0001817	0.1197	0.2406	–	6.386e-05
Control	6.386e-05	6.203e-05	6.203e-05	6.34e-05	6.386e-05	–

P-values inferior at 0.05 in a column show a significantly different between two treatments (plant extracts) or one with the control.

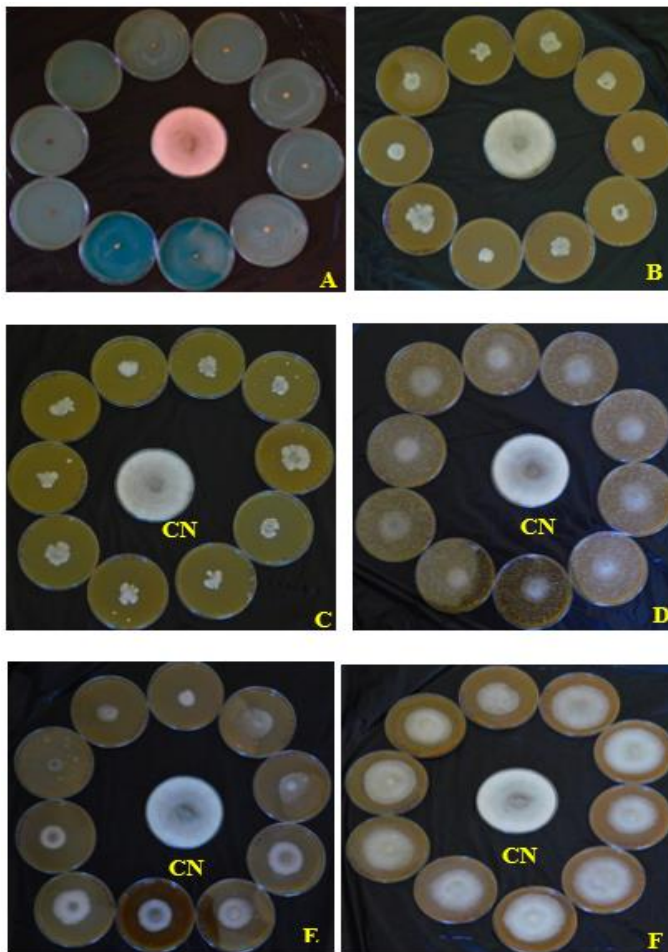


Figure 3: Antifungal activity of five plant extracts and Mancozeb on the mycelial growth of *L. pseudotheobromae* in ten Petri dishes. (A: Mancozeb, B: *A. indica*; C: *C. procera*; D: *G. arborea*; E: *J. curcas*; F: *E. camaldulensis*; CN: Petri dish control).

DISCUSSION

Efficacy of extracts from the five plant species tested

The crude aqueous extracts from all five plant species showed antifungal activities against the two *Lasiodiplodia* species. Their antifungal activities were lower than those of the reference antifungal drug used, Mancozeb which showed 100% inhibition. Chemical fungicides are quite expensive and leave drastic implications for human health and the environment; so we have alternatively chosen the botanical extracts under *in vitro* conditions to find out the control of *L. theobromae* and *L. pseudotheobromae*, pathogens responsible of the disease. Findings from this study were in agreement with previous researchers, who reported that most the mycelial growth of most isolates of *L. theobromae* and *L. pseudotheobromae* from mango and other crops were significantly inhibited by leaf extracts from various medicinal herbs and spices (Rahee et al., 2018). About, acalypha (*Acalypha hispida*), siam weed (*Chromolaena odorata*), aidan (*Tetrapleura tetraptera*), and neem (*Azadirachta indica*) were reported to significantly inhibit *L. theobromae* isolates of cashew inflorescence blight in Nigeria (Dele and Abiodun, 2015). In the same country, *in vitro* tests showed antimicrobial activity of crude extracts against *Botryodiplodia*, one of fungal pathogens of yam. The cold water extracts of *J. curcas* had inhibitory effects against this pathogen with range of inhibition colony diameter of : 6.3 - 16.0 mm (Opara and Nwokocha, 2015). In Pakistan, *in vitro* evaluation of the effectiveness of the extract of four plants species against the mycelial growth of *B. theobromae* revealed that safeda (*E. camaldulensis*) and Neem extracts were the most effective while garlic (*Allium sativum*) and onion (*Allium cepa*) extracts were comparatively and statistically less effective (Sahi et al., 2012). Moreover, comparative analysis showed that *D. stramonium* and *E.*

camaldulensis extracts most efficiently reduced the growth of *L. theobromae* causing stem end rot of mango in Pakistan, in comparison to Aloe-vera extract (Ullah et al., 2017).

In contrast, extracts of *E. camaldulensis* in our study showed the low inhibitory effect (< 38% inhibition) on both target fungi (*L. theobromae* and *L. pseudotheobromae*), suggesting a possible variation in effectiveness of the extract depending to the geographical origine of the plant species. The effectiveness of extracts from these plant species have also been reported on other fungus species indicating that they may have a large spectrum of antifungal activity. Indeed, Ahmad et al. (2016) revealed the efficacy of *Eucalyptus globulus*, *C. procera*, *Melia azedarach*, *Datura stramonium* et *Acalypha indica* in reducing the mycelial growth of *Alternaria alternata*, seed-borne fungal isolated from barley. Following an *in vitro* study, crude extracts of leaves of six different plants which *A. indica* (A.) and *E. camaldulensis* showed antifungal activity against five pathogenic fungi (*Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani*) (Hussain et al., 2015). Pharmacological research reviewed that *G. arborea* possess various medicinal properties and biological activities including antidiuretic, antidiarrhoeal, antipyretic, antianalgesic, antioxidant, antidiabetic, antihelmintic, antibacterial, antifungal, cardioprotective, insecticidal, antiulcer, gastro-protective, anticancer, antihyperlipidemic and immunomodulatory activity (Arora and Tamrakar, 2017).

Efficacy of concentrations of extracts and phytochemical constituents

Growth reduction in *L. theobromae* and *L. pseudotheobromae* was depending to the type of plant extract, the concentration of extract used and as well as the duration of incubation. Findings of this study revealed that

the effectiveness of extracts increased as their dose increased. This is in agreement with earlier report (Ehiobu and Ogu, 2018). They reported that *M. esculenta* leaf extracts at concentrations (25 g/l and 50 g/l) generally demonstrated least antifungal activities against *B. theobromae* and *A. niger*, their activities being significant at 75 g/l.

According to Banso and Adeyemo, (2007) the actions of the antifungal substances present in the plant extracts were fungistatic at lower concentrations but became fungicidal at higher concentrations. Antifungal activities expressed by the five botanicals against the two *Lasiodiplodia* species in the present study suggest that they possess phytochemical constituents against these fungal pathogens. According to Alessandra et al. (2004), plant secondary metabolites have great potential as a source of effective antifungal agents. About, *A. indica*, contains at least 35 biologically active principals of which triterpenoides eg, nimbin nimbidine and azadirachtin reported are the most active insecticidal or antifungal ingredients (Mordue et al., 2000 ; Brahmachari, 2004). A phytochemical analysis of aqueous extracts of leaves of *C. procera* revealed the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, and saponins glycosides (Hassan et al., 2006). Similar study realised on aqueous extracts of leaves of *J. curcas* by Opara and Nwokocha (2015) allowed to detect bioactive compound such as alkaloid, saponin, flavonoid, phenol, tannin, phytate and HCN. Some constituents from extract of *G. arborea* like 7'O-ethyl arboreol, paulownin, gmelinol, epieudesmin and B-sitosterol have been reported to exhibit antifungal activity against *Trametes versicolor* and *Fomitopsis palustris*, *Aspergillus niger*, *Penicillium notatum* and *Candida albican* (Arora and Tamrakar, 2017). The crude leaf and stem bark extracts of *G. arborea* contains also bioactive compounds such as alkaloids, saponins, carbohydrates, phenolics,

anthraquinone and tannins recorded in the others plants species tested in this study. The secondary metabolites screening of *E. camaldulensis* leaf extracts from Nigeria confirmed presence of tannin, saponins, and cardiac glycosides (Ayepola and Adeniyi, 2008). The chemical constituents such as Tannins, saponins, terpenoids, anthraquinone, glycoside, alkaloids, flavonoids, steroids and reducing sugars are secondary metabolites of plant that have been reported to serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Adeshina et al., 2009; Ogutu et al., 2012).

The presence of cardiac glycosides and steroids have been documented to inhibit the many microbes and found to possess antioxidant potentials (Mujeeb et al., 2014). The differences in the toxicity of different extracts of the five plants species studied could be attributed to the presence of the active principles that are extracted by different solvents, which may be influenced by several factors such plant species, method of extraction and type of extracting solvent (Onzo et al., 2016). Although mancozeb a synthetic fungicide consistently induced 100% inhibition on the two *Lasiodiplodia* species irrespective of the concentration used, it is advice that its use can only be recommended when other methods prove ineffectiveness due to its toxic effect on the environment (Markson et al., 2012). Thus, the extracts of the five plants species have to be tested *in vivo* on mango seedling inoculated with the two *Lasiodiplodia* species following fields trials. The extracts with high could be used more efficacy could be use as alternative to chemicals in the management of these fungal pathogens associated with mango decline.

Conclusion

The present study was carried out under *in vitro* condition to find out the most effective and eco-friendly approach to manage the

mango decline caused *L. theobromae* and *L. pseudotheobromae*. The result proved that plants may contain fungitoxic principles against *Lasiodiplodia* species. *G. arborea*, *C. procera*, *A. indica* and *J. curcas* were more effective on *L. theobromae* and *L. pseudotheobromae* compared to *E. camaldensis* plants extracts at various concentrations. These plants could therefore be formulated and used as alternative to chemicals in the management of fungal pathogens of mango decline since they have less adverse environmental effects, are easily available and less difficult to prepare compared to the use of synthetic fungicides which are very costly and harmful to human and environment. However, efficacy trials of these extracts must be carried out on mango plants inoculated in a greenhouse followed by field trials to detect the most effective.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

DZO, WI and DF made a substantial contribution to the design of the work. They contribute to the acquisition, analysis or interpretation of data. All authors brought a contribution to the elaboration of the manuscript and revised it critically for important intellectual content, and approved to be published.

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