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Antifungal potential of some plant extracts against threepost-harvest fungal pathogens of avocado (*Perseaamericana* Mill.) fruits

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Abstract
Thestudy was conducted with the objective of evaluating the antifungal activity of *Carica papaya, Cupressus lusitanica, Erigeron floribundus* and *Euphorbia hirta* extracts against *Colletotrichum gloeosporioides, Botryosphaeria dothiorella* and *Cercospora purpurea in vitro* and for controlling their development on artificially inoculated avocado fruits. Leaves of *C. papaya,* young branches of *C. lusitanica* and aerial parts of *E. Floribundus* and *E. hirta* collected in Dschang, Cameroon, were dried under shade and extracted using ethanol and distilled water. All the extracts showed fungicidal effect especially ethanol extracts.The *in vitro*test showed that ethanol extracts of *C. lusitanica* and *C. papaya* completely inhibited the growth of *B. dothiorella* and *C. gloeosporioides* at concentration of 4mg/ml compared tothose of *C. purpurea* which was completely inhibitedthese species at 5.5 mg/ml.. The results of *in vivo*testshowed that fruits inoculated with different pathogens and treated with the extracts of *C. lusitanica* and *C. papaya* did not develop any lesion 5 days after inoculation. Extracts from *E. Floribundus* lost their *in vivo* efficacy. These results suggest that extracts of *C. lusitanica* and *C. papaya* possess biofungicidal potential, which can suitably be exploited to control avocado fruit rots.

Keywords: Avocado fruits, post-harvest fungi, plant extracts, antifungal activity

1. Introduction
Avocado (*Persea americana* Mill.) is one of the most important fruit crops in the tropics. Economically, it provides food and source of income to millions of people all over the world. In Cameroon, avocado production influences the economy of the rural population, particularly that of the West region where it constitutes one of the main sources of income [1]. Avocado is a nutrient rich fruit; the ripe fruit contains vitamin A, B, C, minerals, potassium, phosphorus, magnesium and iron. It also contains high levels of lipophilic and bioactive plant chemicals including vitamin E, carotenoids and sterols that display antioxidant and radical scavenging activities [2]. Although avocado fruit has an excellent nutritional value; it suffers a number of post-harvest pathogens which can affect its quality and life span, resulting *in* enormous losses. These post-harvest losses are attributed to many factors among which rots due to fungal organisms *that*limit the commercial value of the fruit.Avocado fruit rots are commonly caused by *Colletotrichum acutatum*, *C. gloeosporioides*, *Botryosphaeria parva*, *B. dothiorella* and *Phomopsis* sp. [3, 4]. These fungi infect through the sides of the fruit or through the cut pedicel and the symptoms only express when the fruit begins to ripen after harvest.
Synthetic fungicides have been currently used for the control of post-harvest diseases. However, the health of the consumer, the undesirable effects, the development of fungicide resistantstrains and the high cost of these products have it essential raised up awareness to investigate alternative methods for controlling post-harvest decay of fruits[5, 6]. On the other hand, theapplication of higher concentrations of chemicals in an attempt to overcome diseases on fruits increases the risk of high levels of toxic residues, which is, particularly serious, since avocado fruit is consumed in arelatively short time after harvest [7].
Natural products of plants are less persistent in the environment and are safe for mammals, other non-target organisms [8, 9], and for the control of post-harvest diseases than synthetic products[10]. A number of plant species have been reported to possess natural substances that are toxic to many plant pathogenic fungi [11, 12].

Avocado fruit rot is one of the major diseases of the crop in Cameroon. Some fungi have been identified as being important pathogens of avocado in Cameroon, these are *Colletotrichum gloeosporioides*, *Botryosphaeria dothiorella* and *Cercospora purpurea* and each of these fungi can cause rot lesion on thesefruits [13]. Hence, this study was conducted with the objective of assessing the *in vitro* effect of plant extracts on mycelia growth of *Colletotrichum gloeosporioides*, *Botryosphaeria dothiorella* and *Cercospora purpurea* and to test their *in vivo* efficacy on the development of these post-harvest pathogens on avocado fruits.

2. Materials and Methods

2.1 Isolation of fungal pathogens

Colletotrichum gloeosporioides, *Botryosphaeria dothiorella* and *Cercospora purpurea* were isolated from ripe avocado fruits collected from the local market and presenting rot lesions. The fungi were identified in the PlantPathology Laboratory of the University of Dschang, Cameroon as described by Keuete, 2014 and pure cultureswere maintained on potato dextrose agar (PDA) at 4°C.

2.2 Plant extracts

Fresh leaves of *Carica papaya, Cupressus lusitanica* and aerial part (leaf and stem) of *Erigeron floribundus* and *Euphorbia hirta* were collected in March 2013 from the locality of Dschang, West region of Cameroon**.** Their identification was confirmed through consultation in the Herbarium of the Department of Plant Biology, University of Dschang. Plant parts collected were washed three times with running tap water and rinsed with sterile distilled water. They were separately air-dried at a room temperature and ground in a mortar. One hundred grams of the resulted dried powder were macerated in 500 ml of distilled water or ethanol and mixed thoroughly. The mixture was allowed to restfor 48 hours for aqueous extract and the supernatant passed through whatman's N°. 1 filter paper to obtain the extract. For ethanolextract, after maceration during 4 hours in a warring blender (Warring International, New Hartford, CT, USA), the macerate was passed through Whatman's N°. 1 filter paper and evaporated using a Rota vapour at 40°C water bath temperature (Heidolph) [14]. Extracts were preserved aseptically in a brown bottle at 4°C until further use [15].

2.3 *In vitro*antifungal activity of plant extracts

The *in vitro* antifungal activity was assessed according to the agar dilution method [16] on PDA (Difco). Plant extracts were dissolved in dimethylsulphoxide (DMSO) and diluted to give serial dilutions that were incorporated into growth medium. The resulting concentrations were 1, 2.5, 4, 5.5 and 7 mg/ml for ethanol extracts and 5, 10, 15, 20 and 25 mg/mlfor aqueous extracts. PDA medium supplemented with different concentrations of the extracts were inoculated with 6-mm diameter (plugs) of the test pathogen cut from the margin of 7-day-old cultures. The plates were incubated in duplicates over a period of 8 days for *B. dothiorella* and 10 days for *C. gloeosporioides* and *C. purpurea* at 20 ± 2°C. The radial mycelia growth was measured daily and the fungi toxicity was expressed as percentage inhibition of radial mycelia growth using the formula below according to [17]:

$$P_I = \frac{DT - D}{DT} \times 100$$

Where D_T and D, are the radial mycelia growth measurements in the control and treatment plates respectively.

In order to distinguish between fungicidal and fungi-static activity of the selected plant extract against the test pathogen, the mycelia plugs that did not show any growth were transferred to a freshly poured PDA plate and incubated for 7 days at 20 ± 2°C to observe the recovery of the growth. The fungicidal effect was classified as an absence of growth whereas any observed growth was classified as fungi-static. The plant extracts that showed fungicidal effect (*in vitro*) were further used to control these post-harvest pathogens on inoculated fruits.

2.4 *In vivo* assay of plant extracts

Based on the results of *in vitro* test, one concentration (20 mg/ml) of aqueous extract and two concentrations (5.5 and 7 mg/ml) of ethanol extracts were used for this test. Conidial suspension were prepared from pure culture of *C. gloeosporioides*, *B. dothiorella* and *Cercospora purpurea* and adjusted to a concentration of 5 x 10^4 conidia/ml using heamocytometer. Apparently healthy avocado fruits collected from the market in Dschang were washed with tap water, dried and surface-sterilized by alcohol. These fruits received simultaneously on the epicarp 50 µl of conidial suspension and 50 µl of each plant extract at the above concentrations that showed fungicidal activity during *in vitro* test [18].

Inoculated and treated fruits were arranged in plastic plates containing cotton soaked with sterile distilled water to maintain humidity during the experiment. Five days after inoculation at ambient temperature (24±2°C), the lesion areas developed on the fruits were evaluated using a graph paper. A completely randomized design was used with 4 replications and theexperiment was repeated thrice.

2.5 Statistical analysis

Data collected on percentage inhibition and lesion area were subjected to analysis of variance (ANOVA) using SPSS software version 17. The mean values were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

3. Results

3.1 *In vitro* activities of ethanol extracts

Antifungal effects of ethanol extracts of *E. floribundus, E. hirta, C. lusitanica* and *C. papaya* on fungal growth are presented in Table 1. The extracts of four plants with increasing concentrations showed a gradual inhibition of the growth of the post-harvest pathogens (*B. dothiorella, C. gloeosporioides* and *C. purpurea*). However, complete inhibition of mycelia growth of *B. dothiorella* and *C. gloeosporioides* with ethanol extracts of *C. lusitanica* and *C. papaya* was observed at concentration 4mg/ml while that of *C. purpurea*was noted at the concentration of 5.5 mg/ml. Also, ethanol extract of *E. floribundus* completely inhibited the growth of *B. dothiorella* and *C. gloeosporioides* at 5.5 mg/ml and that of *C. purpurea* at 7 mg/ml. Concerning ethanol extract of *E. hirta* which seems to be the least active, complete growth inhibition of *B. dothiorella* and *C. gloeosporioides* was observed at 7 mg/ml.

Table 1: Effect of ethanol plant extracts on the percentage inhibition of radial mycelia growth of the fungal pathogens

	B. dothiorella			
Dose (mg/ml)	*E. floribundus*	*E. hirta*	*C. lusitanica*	*C. papaya*
0 (T-)	0.00 ± 0.00^{e*}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
1	19.44 ± 6.42^{d}	6.94 ± 4.05^{e}	58.33 ± 6.55^{c}	65.07 ± 2.68^{c}
2.5	32.53 ± 8.25^{c}	32.00 ± 3.96^{d}	82.93 ± 9.00^{b}	82.72 ± 10.99^{b}
4	54.36 ± 6.52^{b}	54.00 ± 7.43^{c}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
5.5	100.00 ± 0.00^{a}	84.66 ± 6.87^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
7	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
1 (Mancozeb)	100.00 ± 0.00^{a}	100.00 ± 0.00a	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	C. gloeosporioides			
0 (T-)	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}	0100 ± 0.00^{c}
1	57.93 ± 4.62^{d}	47.42 ± 6.19^{e}	70.03 ± 2.81^{c}	88.88 ± 2.99^{b}
2.5	78.37 ± 3.05^{c}	67.65 ± 2.47^{d}	90.07 ± 2.99^{b}	96.82 ± 2.47^{a}
4	95.63 ± 1.23^{b}	79.16 ± 2.14^{c}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
5.5	100.00 ± 0.00^{a}	94.44 ± 1.91^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
7	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
1 (Mancozeb)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	C. purpurea			
0 (T-)	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
1	41.86 ± 2.40^{d}	13.29 ± 5.53^{d}	64.44 ± 1.37^{c}	43.65 ± 9.09^{d}
2.5	59.32 ± 6.36^{c}	34.72 ± 9.69^{c}	75.00 ± 6.76^{b}	74.20 ± 9.67^{c}
4	75.99 ± 5.96^{b}	72.22 ± 5.87^{b}	96.82 ± 2.99^{a}	87.67 ± 6.89^{b}
5.5	94.24 ± 4.54^{a}	77.38 ± 5.74^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
7	100.00 ± 0.00^{a}	97.22 ± 2.99^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
1 (Mancozeb)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Values in the same row followed by different letters are significantly different (P <0.05). Data are means ± SD of three experiments
T- = negative control (Distilled water) ; M = mancozebe.

3.2 *In vitro* effect of aqueous plant extracts

Table 2 summarizes the results of *in vitro* effect of aqueous extracts of *E. floribundus, E. hirta, C. lusitanica* and *C. papaya* on growth of *B. dothiorella, C. gloeosporioides* and *C. purpurea*. The results are presented in percentage of fungal growth inhibition. Mycelia growth of *B. dothiorella*, *C. gloeosporioides* and *C. Purpurea* were significantly (P<0.05) inhibited by aqueous extracts of the three plants. Significant differences (P < 0.05) in mycelia growth between plates supplemented with plant extracts and the negative control supplemented with distilled water were noted. Apart from *C. gloeosporioides* that was inhibited with aqueous extracts of *C. lusitanica* and *C. papaya* at 15mg/ml, complete inhibition was shown with the same extracts on other fungal pathogen at 20mg/ml. With aqueous extracts of *E. floribundus* and *E. hirta*, inhibition of the three fungi (*B. dothiorella*, *C. gloeosporioides* and *C. purpurea*) were observed at 25 mg/ml.

Table 2: Effect of aqueous plant extracts on the percentage inhibition of radial mycelia growth of the fungal pathogens

	B. dothiorella			
Dose (mg/ml)	*E.floribundus*	*E. hirta*	*C. lusitanica*	*C. papaya*
0 (T-)	0.00 ± 0.00^{e*}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
5	12.89 ± 6.25^{d}	3.57 ± 3.14^{e}	31.15 ± 15.63^{c}	59.72 ± 3.78^{c}
10	41.46 ± 5.40^{c}	13.49 ± 3.48^{d}	68.84 ± 13.96^{b}	87.89 ± 2.81^{b}
15	76.58 ± 8.12^{b}	45.63 ± 5.18^{c}	93.84 ± 5.05^{a}	99.00 ± 1.71^{a}
20	96.82 ± 2.99^{a}	89.00 ± 5.18^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
25	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
1 (M)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	C. gloeosporioides			
0 (T-)	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
5	53.57 ± 25.47^{b}	11.11 ± 9.64^{d}	91.01 ± 1.78^{b}	92.26 ± 3.71^{b}
10	78.57 ± 4.72^{a}	38.29 ± 15.12^{c}	98.07 ± 2.09^{a}	99.00 ± 1.23^{a}
15	82.73 ± 22.71^{a}	74.40 ± 2.14^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
20	100.00 ± 0.00^{a}	96.62 ± 5.84^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
25	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
1 (M)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	C. purpurea			
0 (T-)	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}
5	35.31 ± 1.91^{d}	42.26 ± 10.01^{d}	81.54 ± 4.50^{b}	54.34 ± 4.30^{c}
10	44.44 ± 5.40^{c}	55.95 ± 6.76^{c}	82.93 ± 3.90^{b}	66.67 ± 3.14^{b}
15	48.61 ± 0.90^{c}	65.07 ± 3.63^{b}	100.00 ± 0.00^{a}	98.61 ± 2.40^{a}
20	61.90 ± 3.31^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
25	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
1 (M)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Values in the same row followed by different letters are significantly different (P <0.05). Data are means ± SD of three experiments
T- = negative control (Distilled water) ; M = mancozebe.

3.3 Toxicity nature of plant extracts against fungal species.

Transfer of mycelia plugs that did not show any growth unto freshly poured PDA plate without plant extracts, showed fungi-static and fungicidal effects after 2 to 10 days of incubation.

With regards to aqueous extracts at 25 mg/ml concentration, *E. hirta* and *E. floribundus* showed fungi-static effect with *B. dothiorella* and fungicidal effect on *C. gloeosporioides* and *C. purpurea*. Aqueous extracts of *C. lusitanica* and *C. papaya* at 20 mg/ml were fungicidal on *C. gloeosporioides*and *C. purpurea,* and on *B. dothiorella* at 25 mg/ml.

For ethanol extracts, *E. floribundus* and *E. hirta* were fungi-static, but fungicidal effects were observed at 7 mg/ml concentration with *E. floribundus* and *B. dothiorella*. Ethanol extracts of *C. lusitanica* and *C. papaya* at 5.5 mg/ml were fungicidal on *B. dothiorella* and *C.purpurea* while on *C. gloeosporioides,* a fungicidal effect was observed respectively at 7 mg/ml and 5.5 mg/ml concentrations.

3.4 *In vivo* activities of plant extracts

The results of *in vivo* activity of plant extracts on the development of *B. dothiorella, C. gloeosporioides* and *C. purpurea* are presented on table 3. Apart from the inoculated avocado fruits that received a deposit of extracts of *E. floribundus* and the control fruits where lesions were observed, other fruits treated with aqueous and ethanol extracts of *C. lusitanica* and *C. papaya* did not show any lesion. However, fruits treated with aqueous and ethanol extracts of *E. floribundus* developed lesions occupying a surface area of 79 and 91 mm^2 which were significantly lower than those from the control fruits (177 and 359 mm^2).

Table 3: Effect of plant extracts on lesion area developed by different fungal pathogens on fruits of avocado

	B. dothiorella		***C. gloeosporioides***		***C. purpurea***	
	Dose	Lesion area (mm^2)	Dose	Lesion area (mm^2)	Dose	Lesion area (mm^2)
Aqueous extract						
T	0	$180.00 \pm 0.72^{a*}$	0	359.00 ± 1.59^{a}	0	177.00 ± 0.96^{a}
E. floribundus	/	/	25	91.00 ± 0.37^{b}	25	79.00 ± 0.31^{b}
C. lusitanica	20	0.00 ± 0.00^{c}	15	0.00 ± 0.00^{c}	15	0.00 ± 0.00^{c}
C. papaya	20	0.00 ± 0.00^{c}	20	0.00 ± 0.00^{c}	20	0.00 ± 0.00^{c}
Mancozeb	1	0.00 ± 0.00^{c}	1	0.00 ± 0.00^{c}	1	0.00 ± 0.00^{c}
Ethanol extract						
T	0	180.00 ± 0.72^{a}	0	359.00 ± 1.59^{a}	0	177.00 ± 0.96^{a}
E. floribundus	7	101.00 ± 0.25^{b}	/	/	/	/
C. lusitanica	5.5	0.00 ± 0.00^{c}	7	0.00 ± 0.00^{c}	7	0.00 ± 0.00^{c}
C. papaya	7	0.00 ± 0.00^{c}	5.5	0.00 ± 0.00^{c}	5.5	0.00 ± 0.00^{c}
Mancozeb	1	0.00 ± 0.00^{c}	1	0.00 ± 0.00^{c}	1	0.00 ± 0.00^{c}

Values in the same row followed by different letters are significantly different ($P < 0.05$). Data are means ± SD of three experiments

T = negative control (Distilled water) ; / = Extract not applied

4. Discussion

The effectiveness of the plant extracts varies according to the fungus species, plant species and extraction methods. The results revealed that these plant extracts caused significant inhibition in the mycelia growth of the three fungi tested with ethanol extracts being more effective than aqueous extracts. The difference observed in fungi toxic activity of the extracts might be due to the solubility of the active compounds in water and ethanol or the presence of inhibitors to the fungitoxic agents. These plants could contain some antifungal metabolites. The presence of antifungal substances in several extracts had been reported to inhibit *in vitro* fungal radial growth by several authors [11, 19].

The growth inhibition percentages of different fungi by plant extracts proved to be dependent on concentration, type of extract and the plant tested.

Results obtained from *C. papaya* extracts are in agreement with previous studies that showed the antifungal activities of this plant against fungal species responsible of yam tubers rot, groundnuts rust and taro blight [20, 21, and 22]. Also, results obtained with *C. lusitanica* extract are similar to those reported by [23, 24] which showed that this extract inhibited the development of *Phythophtora megakarya* on cocoa pods as well as *P. Colocasiae* on detached leaves of taro respectively.

The weak antifungal activity of *E. floribundus* and *E. hirta* extracts observed in this work corroborates with the results of [25] which showed that extracts from these plants could not completely inhibit the growth of certain fungal species.

On avocado fruits, aqueous and ethanol extracts of *C. lusitanica* and *C. papaya* showed comparable effects with that of mancozeb by preventing the development of fungal species tested. Results obtained with *C. papaya* extract are in agreement with those obtained by [26], who showed that this extract would reduce the frequency of post-harvests fungi on avocado fruits in storage. Similar results were reported by [20] which showed that *C. papaya* extracts could inhibit fungi responsible for yam tubers rot in storage. Extracts of *C. lusitanica* and *C. papaya* may contain some active compounds which could inhibit the development of *B. dothiorella, C. gloeosporioides*and *C. purpurea* and protect avocado fruits against fungal invasion. These compounds might also contribute to stimulate the avocado fruit's defence system. Lesions were observed on the fruits treated with *E. floribundus* extracts after 5 days of incubation. This fungal development could be explained by the decrease of fungal activity by *E. floribundus* due to the composition of fruit's epicarp which can prevent good diffusion of the extract.

5. Conclusion

This work has revealed exploitable potential of extracts of *Carica papaya* and *Cupressus lusitanica* as prospective source of compounds effective against *Colletotrichum gloeosporioides*, *Botryosphaeria dothiorella* and *Cercospora purpurea*. These extracts also inhibited fungal development on artificially inoculated fruits and could be used to extend the life span of avocado fruits. There is no doubt that these plant extracts possess antifungal agents, however further work should focus on the confirmation of the active ingredient in the plant extracts of *Carica papaya* and *Cupressus lusitanica* for further use in the management of the three fungal pathogens on post-harvest infections of fruits.

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