Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Paper

Activity of extracts from three tropical plants towards fungi pathogenic to tomato (*Solanum lycopersicum*)

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Summary. Antifungal properties were assessed of water and ethanol extracts from the pan-tropical plants *Oxalis barrelieri* L., *Stachytarpheta cayennensis* L., and *Euphorbia hirta* L. against *Fusarium oxysporum* f. sp. *vasinfectum*, *Alternaria solani* Sorauer, and *Rhizoctonia solani* Kuhn. The plant extracts inhibited fungal growth *in vitro* at 1.25-20 mg mL^{-1}, and the degrees of inhibition increased in a dose-dependent manner. Ethanol extracts from the plants inhibited fungal growth by 80-100%, while water extracts showed less antifungal activity, with maximum growth inhibition of 62%. Growth inhibition from ethanol extracts was two- to three-fold greater than for water extracts at equivalent concentrations. Antifungal activity of the extracts varied with their content and composition of phenolics, flavonoids, tannins, and alkaloids. In greenhouse experiments, spraying tomato plants (*Solanum lycopersicum* L.) with ethanol extract from *E. hirta* at 2.5 mg mL^{-1} did not cause phytotoxicity, and increased plant size, when compared to untreated plants. Spraying *E. hirta* ethanol extract on tomato plants infected by *R. solani* reduced disease severity up to 80%, when compared to non-sprayed plants. These results demonstrate potential of leaf extracts from *E. hirta*, *O. barrelieri*, and *S. cayennensis* as biofungicides for the control of *R. solani*, *A. solani*, and *F. oxysporum*, which are among the most important causal agents of tomato diseases.

Keywords. *Euphorbia hirta*, *Oxalis barrelieri*, *Stachytarpheta cayennensis*, antifungal activity, plant growth promotion.

OPEN ACCESS

Citation: P.N. Mekam, S. Martini, J. Nguefack, D. Tagliazucchi, G.N. Mangoumou, E. Stefani (2019) Activity of extracts from three tropical plants towards fungi pathogenic to tomato (*Solanum lycopersicum*). *Phytopathologia Mediterranea* 58(3): 573-586. doi: 10.14601/Phyto-10891

Accepted: October 11, 2019

Published: December 30, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Jean-Michel Savoie, INRA Villenave d'Ornon, France.

INTRODUCTION

In Cameroon, tomato is the most important vegetable crop, with production of over 1.18 million tons harvested from 92,626 hectares in 2016 (FAO, 2017). However, phytopathogenic fungi are responsible for the most prevalent

diseases, including: late blight, caused by *Phytophthora infestans* (Mont.) de Bary; early blight, caused by *Alternaria* spp.; damping-off and seedling blights, caused by *Pythium* spp.; *Rhizoctonia solani* Kuhn and *Verticillium albo-atrum* Reinke & Berthold; and Fusarium wilt and root rot, caused by *Fusarium oxysporum* f. spp. (Jones *et al*., 2014). Furthermore, bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., and bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al.* are also reported to cause severe crop losses in epidemic years (Fontem *et al*., 1999).

Most strategies for pest management rely on frequent application of synthetic chemical pesticides and copper compounds, to avoid yield and quality losses. These may have consequences for human health, the environment, and from development of resistant pathogen strains, due to continuous use of the same available chemicals (Ishii and Hollomon, 2015; Lucas *et al*., 2015). For these reasons, it is important to identify new effective strategies for disease management that pose reduced risks to human health and the environment.

Biofungicides have emerged as the main alternative to conventional fungicides, and the application of plant-derived products is a potential choice in disease management (Ribera and Zuñiga, 2012; Pusztahelyi *et al*., 2015). Tropical plants are rich sources of bioactive chemicals for the development of biopesticides as safe disease control agents, as reviewed by Suprapta (2016). Examples include papaya anthracnose (*Colletotrichum gloeosporioides*) that can be efficiently managed using extracts from tickberry (*Lantana camara*, *Verbenaceae*) (Ademe *et al*., 2013); and grey mold (*Botrytis cinerea*) of blackcurrant controlled by extracts from hyssop (*Hyssopus officinalis*, *Lamiaceae*) and summer savory (*Satureja hortensis*, *Lamiaceae*) (Sesan *et al*., 2015). Several plants are reported to produce diverse arrays of low molecular mass antimicrobial compounds, often called 'natural products'. These may also play important roles in plant physiology. Some of these compounds may provide antimicrobial potential and beneficial effects on plants, such as early seed germination, plant growth promotion, improved crop yield, and increased tolerance to abiotic and biotic stresses (Wink, 2010). The compounds can also enhance postharvest shelf-life of perishable products (Ji *et al*., 2005; Yang *et al*., 2011; Kharchoufi *et al*., 2018; Scavo *et al*., 2019).

Many local plants commonly present in several Cameroon provinces, including *Oxalis barrelieri* L., *Stachytarpheta cayennensis* L., and *Euphorbia hirta* L., have been gaining attention, based on their ethnobotanical uses, phytochemical and pharmacological properties, and their easiness to be cropped in tropical areas. *Euphorbia hirta* has been chemically studied and found to possess antifungal activity against *Fusarium moniliforme* Sheldon and *Phoma sorghina* Saccardo (Karanga *et al*., 2017). *Oxalis barrelieri* plant extracts showed inhibitory effects on mycelium growth and conidia germination of *F. oxysporum* and *P. infestans* (Dakole *et al*., 2016). Plant extract of *S. cayennensis* inhibited the growth of different bacteria (Okoye *et al*., 2010).

Effects of plant extracts on fungal pathogens of tomato have not been studied. The present study aimed to: i) examine the antifungal activity of water and hydro-ethanolic extracts of *E. hirta*, *O. barrelieri* and *S. cayennensis* against three major phytopathogenic fungi affecting tomato (*i.e. R. solani*, *A. solani*, and *F. oxysporum*); ii) determine the phytochemical composition of these extracts; and iii) assess the growth promoting and protective effects of the *E. hirta* ethanol extract on tomato. Once antimicrobial activity is confirmed, such extracts or their components may have potential to be developed as innovative agrochemicals, for implementing sustainable pest management in organic and integrated tomato production.

MATERIALS AND METHODS

Plant material

Three pan-tropical plant species, *Oxalis barrelieri* L. (*Oxalidaceae*), *Stachytarpheta cayennensis* L. (*Verbenaceae*) and *Euphorbia hirta* L. (*Euphorbiaceae*) were collected from a local area (Central Region, Yaoundé-Mbankomo, Cameroon) (Figure 1). These species were chosen based on their previously studied phytochemical properties (Senthikumar, 2018). They were grown until the flowering stage and harvested. After harvesting, the plant material was shade-dried for 2 weeks, and the dried leaves were milled into powder.

Tomato seeds 'Leader F1', obtained from ISI Sementi, Fiorenzuola d'Arda, Italy, were sown and transplanted in a greenhouse into trays containing a commercial soil mix (Dueemme Marketing srl.). The greenhouse was maintained at a constant temperature of 27°C, with a 16 h photoperiod each day. Tomato plants were used for experiments 3 weeks after sowing, at the five to six true leaf stage.

Fungus strains

Fusarium oxysporum f. sp. *vasinfectum*, strain FUSITS04 and *Alternaria solani* Sorauer, both originat-

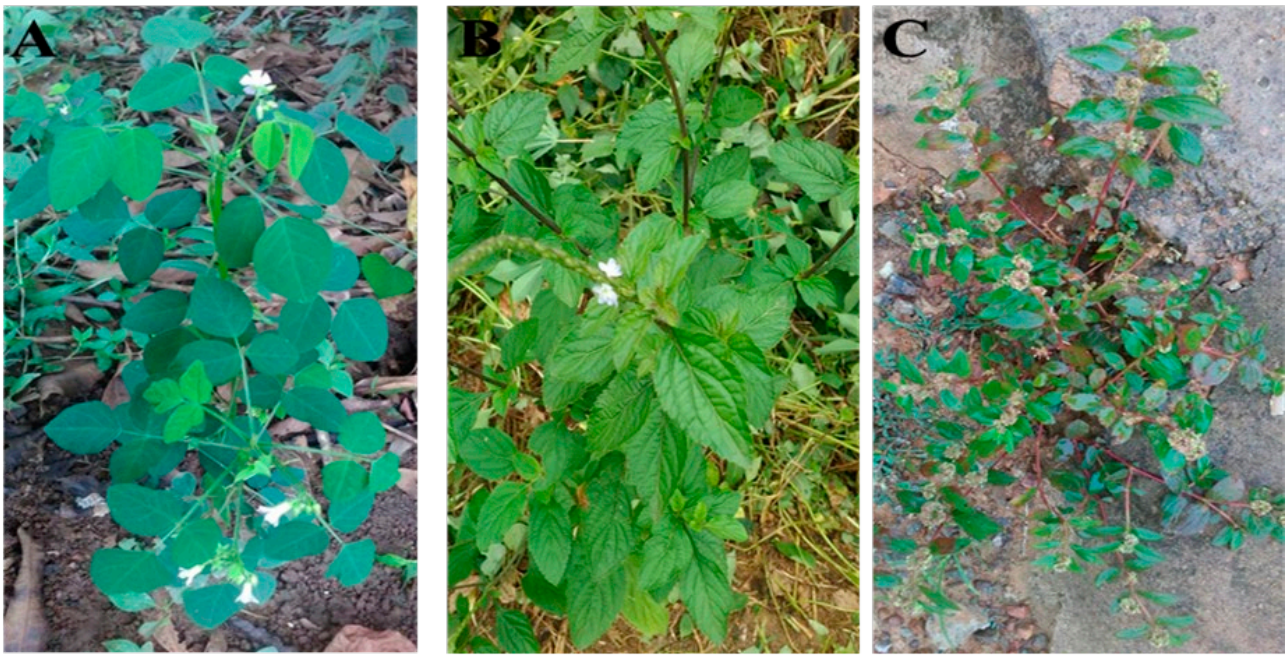

Figure 1. Pan-tropical plants examined in the present study: *Oxalis barrelieri* L. (A), *Stachytarpheta cayennensis* L. (B), and *Euphorbia hirta* L. (C). These plants are shown at the beginning of the flowering stage, during which they were harvested and dried.

ing from Cameroon, and *Rhizoctonia solani* Kuhn (courtesy of P. Nipoti, University of Bologna, Italy) were used during the experiments. These fungi were isolated from diseased tomato plants, and were maintained on 3.9% potato dextrose agar (PDA) at 4°C until used for experiments. *R. solani* was also used for greenhouse *in planta* experiments.

Preparation of crude plant extracts

Before extraction, leaf powders were defatted (1:6, w/v) by mixing in 600 mL of anhydrous n-hexane under continuous stirring at room temperature (21 ± 1°C) for 24 h. After filtration through fine cloth, each defatted plant residue was hexane-evaporated in a laminar flow cabinet. Extraction of the plant residues was achieved using either distilled water or 70% hydro-ethanol solution. Plant material was soaked (1:6, w/v) in 600 mL of distilled water or in 600 mL of ethanol solution under continuous stirring for 24 h, followed by filtration through Whatman No. 1 filter paper (11 µm pore size) and centrifugation at 5,200 × *g* for 10 min. The supernatants were then collected and the solvents evaporated overnight (12 h) in a ventilated oven at 50°C. The dried pellets obtained were designated as: WE*ox* (water extract of *O. barrelieri*), EE*ox* (ethanol extract of *O. barrelieri*), WE*st* (water extract of *S. cayennensis*, EE*st* (ethanol extract of *S. cayennensis*), WE*eu* (water extract of *E. hirta*), or EE*eu* (ethanol extract of *E. hirta*). The extracts were stored at 4°C until used.

Effects of plant extracts on fungus growth in vitro

Effects of different water and ethanol extracts on growth of fungi were assayed *in vitro* on PDA agar plates, amended with different concentrations of the plant extracts, using the supplemented agar method described by Rios *et al.* (1988).

Five increasing amounts of each plant extract, from 0.125 to 2 g, were added to flasks containing 100 mL of PDA medium before autoclaving (at 121°C for 15 min). This gave final concentrations of each extract in poured PDA plates of 1.25, 2.50, 5, 10, or 20 mg mL^{-1}. PDA plates without extract additions were used as experimental controls. Each agar plate was then inoculated with a 5 mm diam. mycelium plug taken from the margin of a 7-d-old culture, and kept in an incubator at 27°C. Growth was assessed after 7 d, by measuring two opposing diameters of the fungus colony. Growth inhibition relative to the controls was calculated according to the following equation:

$$\text{Growth inhibition (\%)} = \frac{D - d}{D} \times 100$$

where, D = colony diameter in the control PDA plate, and d = colony diameter in the amended PDA plate.

Each fungus-extract combination was replicated in five plates, and all experiments were independently repeated three times.

Greenhouse in planta *experiments*

Application of plant extract and fungus inoculation

To perform *in planta* experiments, the most active ethanol extract of *E. hirta* (EE*eu*) was chosen for assessment against *A. solani*. Prior to these experiments, a preliminary assay was carried out on a set of tomato plants to assess for possible phytotoxic effects of EE*eu*. Since no visible phytotoxic effects were observed in this assay after 1 week, an aqueous solution of EE*eu* was used as a spray onto the shoots canopy of tomato plants, at a concentration of 2.50 mg mL^{-1}.

Inoculum of *R. solani* was prepared from 7-d-old cultures grown on PDA by gently blending the mycelium with the agar to obtain an inoculum paste. Inoculation was carried out 48 h after EE*eu* applications, by transferring the tomato plants into new pots containing infested soil-perlite (3:1, v:v). The soil was infected by setting 2.5 g of *R. solani* inoculum paste at the bottom of a hole made at mid-depth in each pot (Logemann *et al*., 1992). The tomato plants were carefully uprooted from their previous pots, partially cut at their root ends and transferred into the inoculated pots, so that the inoculum paste became in contact with the wounded roots. The pots were then arranged in a completely randomized design in a greenhouse maintained at 27°C, under a 16 h photoperiod each day and at 70% relative humidity, and were watered appropriately. The experimental design considered four experimental treatments: i) plants sprayed with EE*eu* (TE); ii) plants inoculated with *R. solani* (TR); iii) plants sprayed with EE*eu*, followed by inoculation with *R. solani* (TE+R); or iv) untreated tomato plants, as the experimental control (TC). Ten replicate plants were used for each treatment, experiments were repeated three times independently.

Evaluation of tomato plant growth promotion and disease

For each treatment, the heights of the treated plants were measured at 7, 14, and 21 d after extract application, to evaluate effects of the extract on plant growth.

Disease severity was recorded for TR and TE+R treatments at 5, 10, 15, and 20 d after inoculation. Severity was scored using a 0-5 scale, were: 0 = healthy plant, 1 = 1-10% of leaves with initial wilts, 2 = 11- 25% of leaves with wilts, 3 = 26-49% of leaves showing wilting and chlorosis, 4 = 50-74% of leaves showing pronounced wilting and development of necrotic areas, and 5 = whole leaves wilting. Severity was calculated according to the following equation:

$$\text{Disease severity (\%)} = \frac{\Sigma dn}{DN} \times 100$$

where, d = severity score; n = number of disease plants with the same severity score; N = total number of the examined plants, and D = the greatest severity score.

Disease reduction was calculated by comparing disease severity observed on plants using the following equation:

$$\text{Disease reduction (\%)} = \frac{S - s}{S} \times 100$$

where, S = disease severity on tomato inoculated with *R. solani* (treatment TR), and s = disease severity on tomato treated with EE*eu* then inoculated with *R. solani* (treatment TE+R).

Analyses of phytochemical contents and antioxidant potential

Preliminary analyses of crude plant extracts were performed to assess their phytochemical compositions. Each extract was analyzed for: i) total phenolic content using the Folin-Ciocalteu reagent, using the method of Singleton *et al.* (1999); ii) total flavonoid content, using the method of Zhishen *et al.* (1999); iii) total tannin content, using the method described by Verzelloni *et al.* (2010); iv) total alkaloid content, using bromocresol green reagent and the method described by Tabasum *et al.* (2016); v) total polysaccharides content using phenol-sulfuric acid after mild acid hydrolysis, using the method of Dubois *et al.* (1956); and vi) total protein content using Bradford's reagent, and Bradford's method (Bradford, 1976).

Antioxidant activity of the plant extracts was measured using the free radical cation 2,2-azinobis-3-ethyl benzothiazoline-6-sulfonic acid, using the method described by Re *et al.* (1999).

Statistical analyses

All data were statistically analyzed using the MaxStat Lite software, version 3.60 (available at: https://maxstat-lite.soft112.com/). Data were subjected to analysis of

variance (ANOVA) and the means comparison carried out using Tukey's multiple range test at $P \leq 0.05$. Principal component analyses (PCA) were performed using the software package Solo, version 8.6.1, (Eigenvector Research, Inc. Manson, WA, USA), considering the analytical properties as variables.

RESULTS

Characteristics of plants extracts

The mean yields of the extracts, based on initial dry biomass of leaves, varied between 5 and 8% (w/w). More precisely, extraction productivity for the different plant extracts was: WE*ox*, 6.2%; EE*ox*, 5.1%; We*st*, 7.4%; EE*st*, 5.3%; WE*eu*, 8.2%; and EE*eu*, 5.6%.

Extracts differed in colour: water extracts were dark brown, and the ethanol extracts were grayish-green.

Antifungal activity of plant extracts

The addition of the plant extracts to PDA medium at all concentrations did not affect medium consistency, but only its colour. Growth of the three phytopathogenic fungi on PDA medium, without addition of plant extracts, reached the following mean colony diameters after 1 week: 8.50 cm for *R. solani*, 7.50 cm for *F. oxysporum* f. sp. *vasinfectum* and 7.38 cm for *A. solani*. Addition of the plant extracts to PDA medium inhibited fungus growth in all of the substrate/fungus combinations, and inhibition was related to extract concentration in the medium (Figure 2A). Increasing concentration of EE*eu* led to complete inhibition of *F. oxysporum*. The same concentration of WE*eu* also inhibited growth of *F. oxysporum*, but to a lesser extent than for EE*eu* (Figure 2B). The tested plant extracts consistently reduced mycelium growth of *R. solani*, *F. oxysporum*, and *A. solani*.

The ethanol extracts gave greater antifungal activity than the water extracts. As little as 1.25 mg mL^{-1} of ethanol extracts from the three plants were sufficient to inhibit fungus growth from 10 to 28% (depending on fungus). Between 10 to 20 mg mL^{-1} of ethanol extract, growth inhibition was 90–100% (Figures 3A, 4A, and 5A). Therefore, the ethanol extracts in sufficient concentration completely inhibited growth of all three fungi. At low doses, water extracts from all three plants also inhibited fungus growth by 2 to 28% (depending on fungus) but differently from the ethanol extracts. Growth inhibition from the water extracts was never as great as with the ethanol extracts (Figures 3B, 4B and 5B). This confirms the greater antifungal activity of the ethanol than water extracts.

Fungus growth inhibition was dose dependent for all the fungus/extract combinations, but the inhibitory effects of extracts varied for extracts from the different plants. In general, extracts from *E. hirta* gave greater inhibition than those from *S. cayennensis*, which were more active than the extracts from *O. barrelieri*.

An effect was observed on the morphology of *F. oxysporum* grown on PDA plates amended with WEs. Mycelium colour and texture were greatly modified on amended PDA plates, as shown in Figure 6. These changes in morphology were not apparent for the other two fungi growing on WEs-amended media.

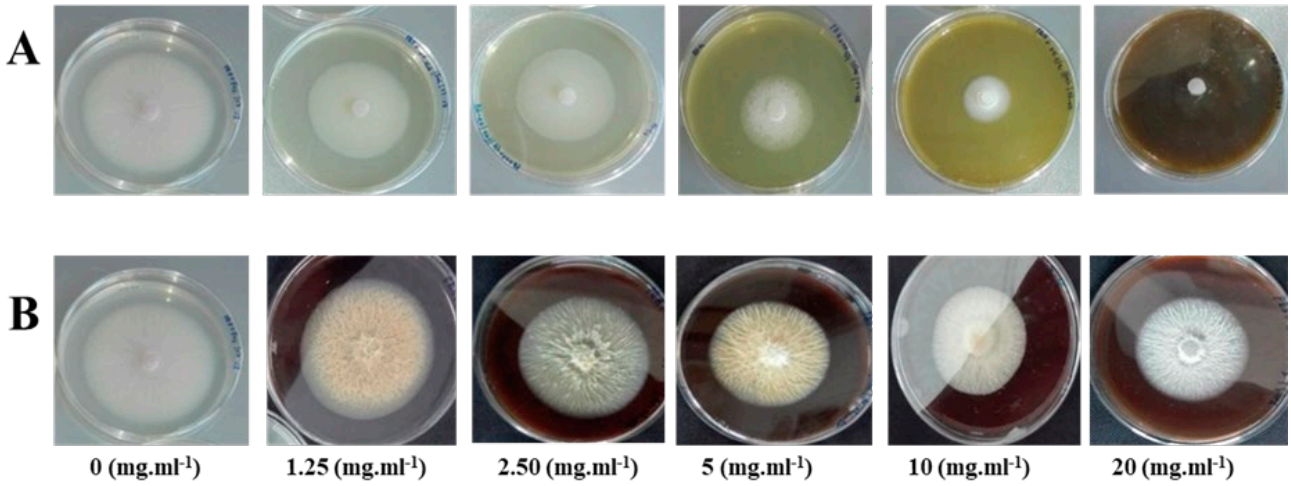

Figure 2. Inhibition of mycelium growth of *Fusarium oxysporum* f. sp. *vasinfectum* after 7 d on PDA supplemented with increasing concentrations of ethanol extract (A) and water extract (B) from *Euphorbia hirta*.

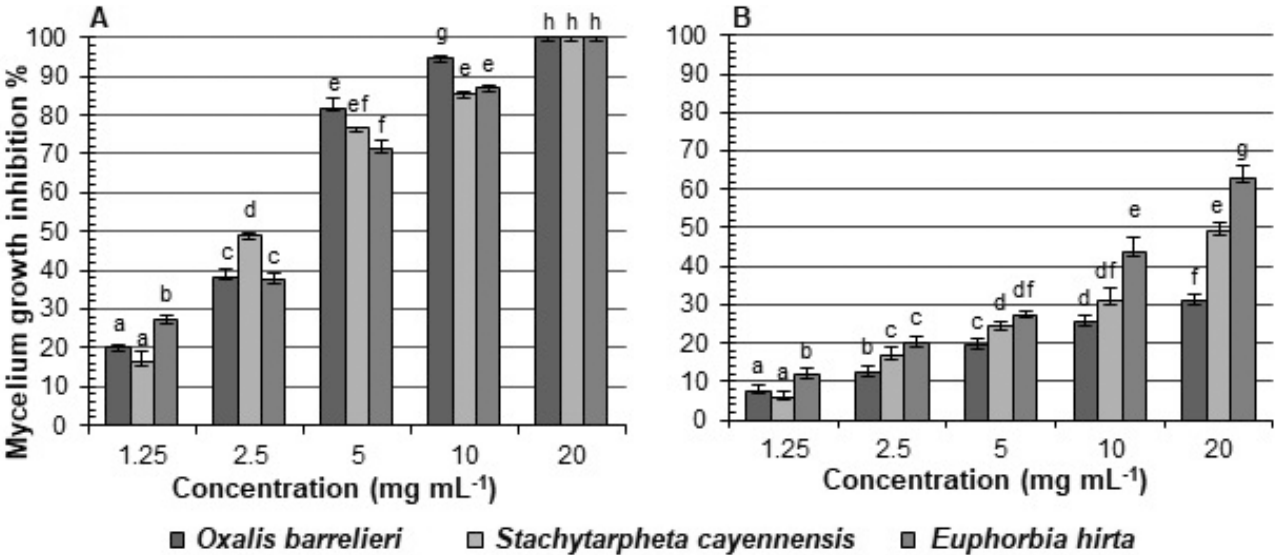

Figure 3. Mycelium growth inhibition (%) of *Fusarium oxysporum* f. sp. *vasinfectum* at 1.25, 2.50, 5, 10 or 20 mg mL^{-1} concentrations of the ethanol extracts (A) or water extracts (B) for extracts from three different plant species. Data are means ± standard deviation from three experiments, each with five replicates. At each extract concentration, the means accompanied by different letters are significantly different, according to ANOVA paired with Tukey's tests ($P \leq 0.05$).

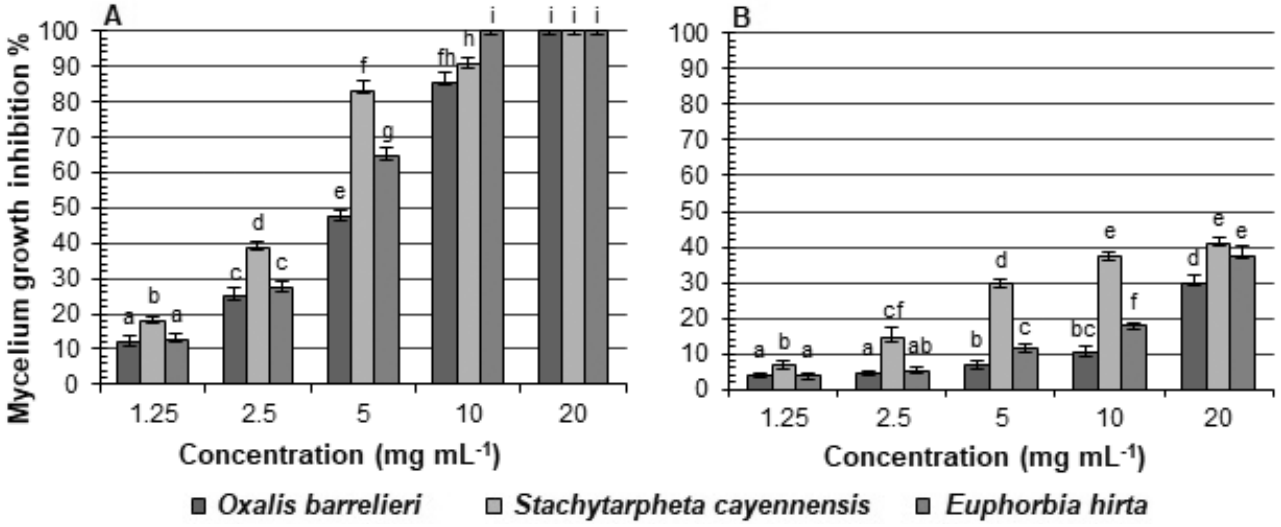

Figure 4. Mycelium growth inhibition of *Rhizoctonia solani* at 1.25, 2.50, 5, 10 and 20 mg mL^{-1} concentrations of the ethanol extracts (A) and water extracts (B) from three plant species. Data are means ± standard deviation from three experiments, each with five replicates. At each concentration, the means accompanied by different letters are significantly different, according to ANOVA paired with Tukey's tests ($P \leq 0.05$).

Effects of Euphorbia hirta *ethanol extract on tomato plant height*

The height of all tomato plants sprayed with EE*eu* (TE) was significantly increased after 7 d (Figure 7). Plant size increased during the following weeks and was greater ($P \leq 0.05$) in EE*eu*-treated plots, when compared to controls. Three weeks after spraying with EE*eu*, mean plant heights from the different extract treatments were: TE, 20.4 cm; TC, 14.5 cm; TE+R, 12.3 cm; and TR. 11.2 cm.

Disease reduction by Euphorbia hirta *ethanol extract*

Data of disease severity and reduction are summarized in Table 1. Tomato plants infected with *R.*

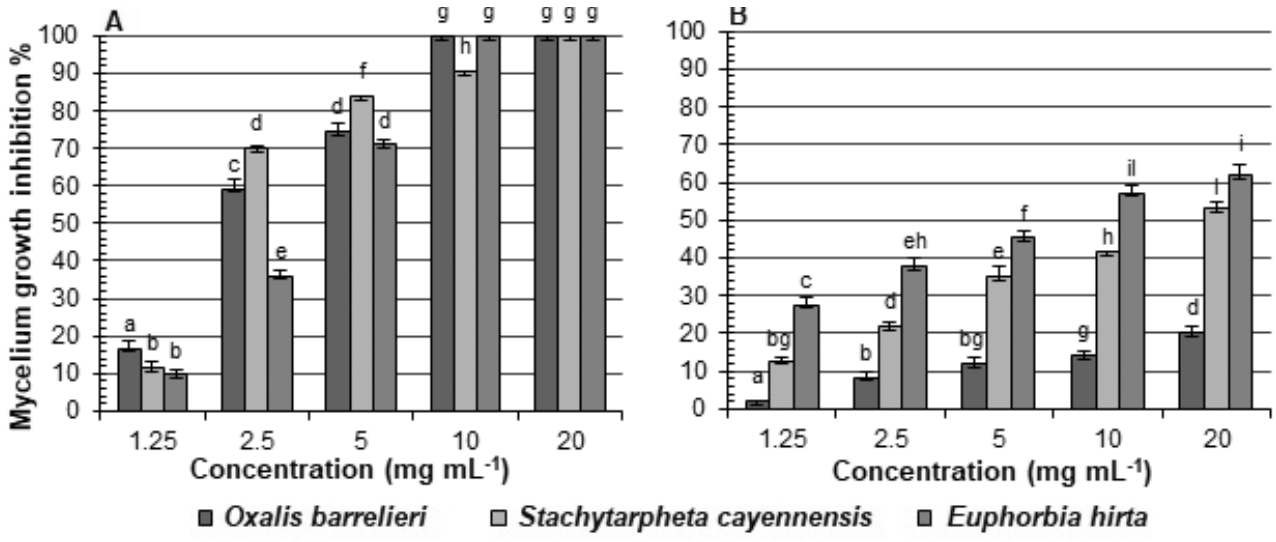

Figure 5. Mycelium growth inhibition of *Alternaria solani* at 1.25, 2.50, 5, 10 and 20 mg mL^{-1} concentrations of the ethanol extracts (A) and water extracts (B) from three plant species. Data are means ± standard deviation from three experiments, each with five replicates. At each concentration, the values accompanied by different letters are significantly different, according to ANOVA paired with Turkey's tests ($P \leq 0.05$).

solani (TR) showed greater disease severity, when compared to EE*eu*-treated plants inoculated with *R. solani* (TE+R). The final disease severity estimates gave 56.7 ± 5% from the TE+R treatment and 80 ± 2% from the TR treatment. This demonstrated that the *E. hirta* ethanol extract efficiently protected tomato plants from *R. solani* infections.

Phytochemical content and antioxidant capacity of the plant extracts

Means values of total phenolic, flavonoid, alkaloid, tannin, protein, and sugar contents and radical antioxidant activity of all the tested plant extracts are presented in Table 2. Except for the alkaloids, whose contents in plant extracts were very similar, the quantification of other chemical components was different for all tested plant extracts. EE*st* had the greatest amounts of phenolics (101.7 ± 11.8 mg gallic acid g^{-1}), flavonoids (33.5 ± 0.7 mg catechin g^{-1}), and tannins (7 ± 1.9 mg catechin g^{-1}). WE*ox* had the greatest amount of proteins (8.1 ± 0.8 mg BSA g^{-1}). The polysaccharide components were greater in water extracts compared to the ethanol extracts, except for EE*st* (179 ± 27.3 mg glucose g^{-1}) and WE*st* (94.8 ± 18.9 mg glucose g^{-1}).

Table 2 shows that WE*ox* and WE*st* were more effective in scavenging ABTS radical cation, with values, respectively, of 262.67 ± 41.48 and 250.33 ± 40.54 mg of L-ascorbic acid g^{-1}. Mean values for the other extracts were: for EE*st*, 168.33 ± 2.36; for EE*ox*, 157.22 ± 4.46; for WE*eu*, 112.53 ± 8.01, and for EE*eu*, 83 ± 26.40 mg of L-ascorbic acid g^{-1}.

The chemical nature of the extracts correlated with the extraction procedures. For instance, *O. barrelieri* was quite productive for total phenolics, when extracted with water (more than double the quantity of phenolics in WE than in EE), whereas *S. cayennensis* and *E. hirta* were much more productive when extracted with ethanol. *Euphorbia hirta* gave the maximum productivity using ethanol for all components, other than total polysaccharides. Total polysaccharides were more abundant in WEs of all three plants. This was expected, since polysaccharides are more soluble in water than in ethanol solutions (Guo *et al.*, 2017). Conversely, total alkaloids and total flavonoids were better extracted with ethanol than water from all three plants: in particular, *E. hirta* was very productive in total flavonoids when extracted with ethanol, compared to water extraction.

The cumulative percentage (74.7%) of the total variance explained by the first two principal components (PC1 and PC2) allowed designing the bidimensional plot represented in Figure 8. The distribution of extracts along PC1 and PC2 shows a clear distinction between ethanol and water extracts, respectively, positive and negative scores on PC1. In order to understand which variables accounted most for this distribution, they were added to the bidimensional plot. The water extracts had negative scores on PC 1 and were characterized by greater polysaccharide contents than the respective ethanol extracts, and the greatest

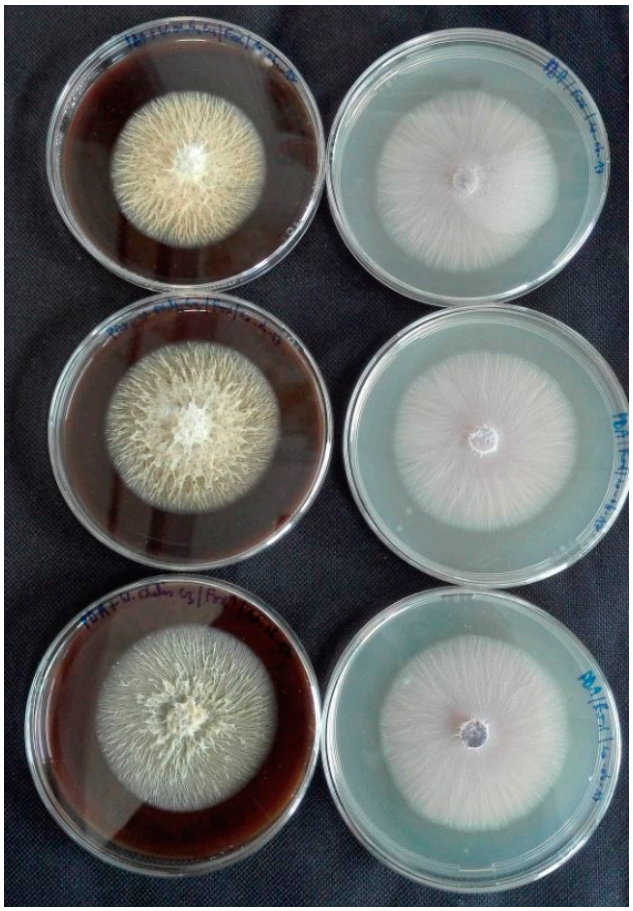

Figure 6. Morphological modification (colour, structure and texture) of *Fusarium oxysporum* f. sp. *vasinfectum* growing on modified PDA amended with water extract from *Oxalis barrelieri*. Left, PDA amended with 5 mg mL^{-1} of water extract; right, PDA without plant extract.

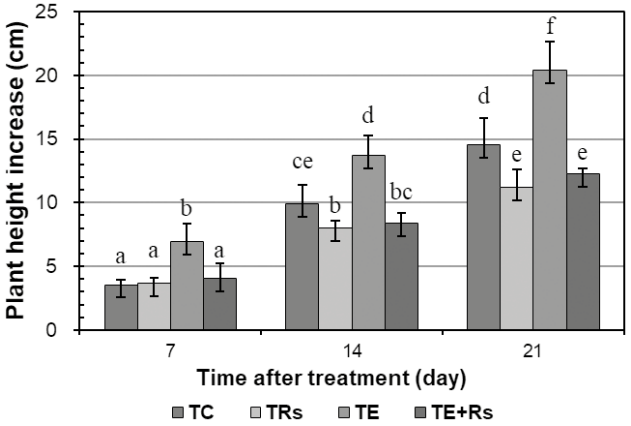

Figure 7. Height (cm) of tomato plants, with or without inoculation of *Rhizoctonia solani*, after treatment with *Euphorbia hirta* ethanol extract, for plants measured at 7, 14 or 21 days after treatment. TC: Negative control, sprayed with water; TRs: positive control, inoculated with *R. solani*; TE: Plants treated with *E. hirta* extract; TE+Rs: Plants treated with *E. hirta* extract and inoculated with *R. solani*. Values are means ± standard deviation, calculated for 30 plants per treatment. Values accompanied by different letters are significantly different, according to ANOVA paired with Tukey's tests ($P \leq 0.05$).

antioxidant activities (WE*ox* and WE*st*, with positive scores on PC 2). Otherwise, ethanol extracts, which were positively linked to PC 1, were characterized by the greatest phenolic, tannin and flavonoid contents (particularly EE*st* and EE*eu*), and were more effective for growth inhibition of the fungi than the respective water extracts, depicted by the positive scores and positive correlation on PC 1.

DISCUSSION

The potential of plant-derived molecules as effective compounds for management of plant pests and diseases raised considerable attention during the last 10-15 years (Reignault and Walters, 2007; Martinez, 2012). This has been particularly important in areas where chemical pesticides may have deleterious impacts on the sustainability of agricultural systems and on food safety (Tripathi and Dubey, 2004; Shuping and Eloff, 2017).

Table 1. Influence of *Euphorbia hirta* ethanol extract on fungal disease caused by *Rhizoctonia solani* on tomato plants.

Treatment	Disease index (%)			
	5 d	10 d	15 d	20 d
R. solani (T_{Rs})	16 ± 2.00^{b}	37.33 ± 3.06^{d}	60.67 ± 7.57^{e}	80 ± 2.00^{f}
Extract + *R. solani* (T_{E+Rs})	8 ± 2.00^{a}	24.67 ± 5.77^{c}	38.67 ± 6.11^{d}	56.67 ± 5.03^{e}
	Disease reduction (%)			
	50.53 ± 6.37	33.43 ± 17.74	35.47 ± 13.59	29.24 ± 4.52

Values are the means ± standard deviation, calculated for 30 plants per treatment.
Different letters indicate significant differences in disease indices, according to ANOVA paired with Tukey's tests ($P \leq 0.05$).

Table 2. Total phenolics, flavonoids, alkaloids, tannins, proteins, polysaccharides content, and antioxidant capacity of three tropical plant extracts from *Oxalis barrelieri*, *Stachytarpheta cayennensis* and *Euphorbia hirta.*

Components	*Oxalis barrelieri*		*Stachytarpheta cayennensis*		*Euphorbia hirta*	
	Ethanol extract	Water extract	Ethanol extract	Water extract	Ethanol extract	Water extract
Total phenolics (mg GAE g^{-1})	34.24 ± 2.65^{b}	70.19 ± 6.80^{d}	101.71 ± 11.84^{e}	49.99 ± 4.62^{c}	71.58 ± 2.34^{d}	24.99 ± 3.87^{a}
Total flavonoids (mg CE g^{-1})	7.52 ± 0.52^{b}	7.03 ± 0.35^{b}	33.54 ± 0.69^{e}	22.95 ± 1.6^{d}	15.16 ± 0.1^{c}	3.47 ± 0.68^{a}
Total tannins (mg CE g^{-1})	0.96 ± 0.44^{ab}	3.39 ± 0.04^{c}	6.96 ± 1.89^{d}	0.94 ± 0.29^{a}	5.00 ± 1.18^{d}	1.73 ± 0.38^{b}
Total alkaloids (mAbs g^{-1})	76.75 ± 21.75^{b}	39.55 ± 12.09^{a}	73.00 ± 12.73^{b}	70.10 ± 16.83^{b}	68.25 ± 11.67^{b}	41.70 ± 18.95^{ab}
Total proteins (mg BSA g^{-1})	4.14 ± 0.28^{b}	8.08 ± 0.82^{e}	5.28 ± 0.22^{c}	4.41 ± 0.27^{b}	6.32 ± 0.27^{d}	1.75 ± 0.11^{a}
Total polysaccharides (mg glucose g^{-1})	46.40 ± 19.24^{a}	83.12 ± 4.01^{b}	94.76 ± 18.88^{b}	179.27 ± 27.25^{c}	59.81 ± 5.07^{a}	84.65 ± 10.71^{b}
Antioxidant activity (mg L-ascorbic acid g^{-1})	157.22 ± 4.46^{c}	262.67 ± 41.48^{e}	168.33 ± 2.36^{d}	250.33 ± 40.54^{e}	112.53 ± 8.01^{b}	83.00 ± 26.4^{a}

Values means ± standard deviation of three replicates.
Uppercase letters in the same row indicate significant differences according to ANOVA paired with Tukey's tests ($P \leq 0.05$).

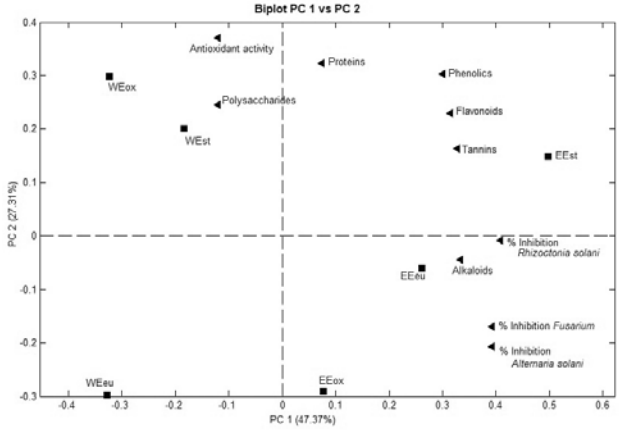

Figure 8. Main biplot (loadings and scores) obtained from principal component analysis for data from ethanol and water extract evaluations, along principal components 1 (PC1) and 2 (PC2). WE and EE indicate, respectively, values for water and ethanol extracts. ox: *Oxalis barrelieri* L., st: *Stachytarpheta cayennensis* L., and eu: *Euphorbia hirta* L. Symbol ▪ depicts the different extracts (scores), whereas ◂ represents the bioactivities and biochemical properties of the extracts (loadings).

In the present study, leaves from three pan-tropical plants, *O. barrelieri*, *S. cayennensis*, and *E. hirta*, were used as potential sources of bioactive molecules against three important phytopathogenic fungi, affecting tomato in Cameroon and worldwide. The three plant species were chosen as sources of possible bioactive/antimicrobial compounds, since they are very common as ruderal herbs in young fallows (Tchiengué, 2012). Additionally, preliminary ethnopharmacological studies showed that these species are relevant as medicinal plants (FAO, 1999).

Our results highlighted the potential fungicidal/fungistatic activity of these plant extracts *in vitro*. Antifungal effects were always more pronounced using hydro-ethanol extracts (EEs) than water extracts (WEs), and were concentration and extract-pathogen-interaction dependent. This confirms the results of Kotze and Eloff (2002), who reported that, in most cases, water extracts had a low antimicrobial efficacy. Therefore, for several plant species, the use of less polar solvents increases the extraction efficiency and concentration of antimicrobial molecules (Eloff, 1998), such as flavonoids and phenolics (Table 2). The complete inhibition of fungal growth was recorded using a concentration ranging from 10 to 20 mg mL^{-1} of EEs obtained from the three plants tested. The antifungal efficacy of such extracts increased linearly ($P \leq 0.05$) in relation to the concentration used, both for EEs and for WEs. This indicates the presence of antifungal molecules in both extracts. The preliminary phytochemical composition of WEs and EEs of the three plants species used in our experiments showed various concentrations of active biomolecules. The concentration of phenolics was greatest in EE*st* and EE*eu* and least in WE*eu*. Conversely, for *O. barrelieri*, more phenolics were found in WE*ox* than EE*ox*. This is possibly due to the diverse nature of phenolics produced by *O. barrelieri* compared, for instance, with *E. hirta* (Mekam *et al.*, 2019). Phenolics (or phenols) are a

large class of compounds classified as simple phenols or polyphenols. They are found in all plants and consist of simple phenols, benzoic and cinnamic acids, coumarins, tannins, lignins, lignans and flavonoids (Khoddami *et al*., 2013). Therefore, it is expected that different plants may produce phenolics in different quantities and of different chemical structures. Phenolics are frequently synthesized in plants in response (or as protection) to stress, such as pathogen infection, insect attack, UV radiation or wounding. Therefore, these compounds have important roles in plant defense (Mandal *et al*., 2010). In most plant species, the key step in phenolic syntheses is the conversion of phenylalanine. This reaction, catalyzed by the phenylalanine ammonia-lyase enzymes, leads to the production of various hydroxycinnamic acids, benzoic acids and derivatives of polyphenols, which are important antifungal biomolecules often found in plants (Raymond Chia and Dykes, 2010).

As highlighted in Table 2, phenolic contents of the different plant extracts were: EE*st* > EE*eu* > WE*ox* > WE*st* > EE*ox* > WE*eu*. Nevertheless, their antifungal activities did not follow this order, since the EEs gave greater antifungal activity than WEs, when tested at equivalent concentrations. EE*eu* gave the greatest antifungal activity, completely inhibiting mycelium growth of *F. oxysporum* f. sp. *vasinfectum* and *A. solani*, at a concentration of 10 mg mL^{-1}. At the same concentration, WE*eu* exhibited moderate antifungal activity, inhibiting mycelium growth of *F. oxysporum* f. sp. *vasinfectum* by 18% and *A. solani* by 57%. In general, hydro-ethanol treatments extracted more (and possibly more diverse) antifungal compounds than water, and this may be related to the less polar nature of ethanol as an extractant, when compared to water. Considering the minimum effective concentration of EEs that completely inhibited fungal growth, the measured EE activity was twice to three times greater than for WEs. These results agree with those of other reports that have showed the efficiency of some hydro-ethanolic plant extracts have the greatest and widest range of *in vitro* activities, resulting in complete inhibition of fungal growth. Galani *et al*. (2013) reported that the EEs of *Ageratum conyzoides* and *Callistemon citrinus* completely inhibited *Phythophthora infestans* at 5,000 ppm, and that of *Ocimum gratissimum* at 10,000 ppm. Dakole *et al.* (2016) reported that the EEs of *Ageratum conyzoides* and *Callistemon citrinus* were the most active for inhibiting radial growth of *Phytophthora infestans*. *Cymbopogon citratus* and *Ocimum gratissimum* were the most active against radial growth and conidia germination of *Fusarium oxysporum* f. sp. *lycopersici* at a concentration of 6,250 µg mL^{-1}. This variability in antifungal activity of extracts suggested that a correlation exist between the nature of plant extracts and their concentrations of active phytochemicals. The amounts, biochemical nature and activity of phenolics (including flavonoids and tannins) and alkaloids contained in the present study extracts was dependent on: i) their solubility in water or ethanol; ii) the absence of inhibitors; iii) their synergism with polysaccharides and proteins present in the extracts; iv) the differences in modes of action; and, v) the structure and biology of the phytopathogenic fungi (Lapornik *et al*., 2005). Therefore, the phenolics present in these plant extracts displayed important and direct inhibition of growth of three tomato pathogens and may be considered as prospective compounds for management of plant diseases. In particular, the extracts from *E. hirta* used in our experiments showed the greatest amounts of phenolic compounds, mainly gallotannins, and hydroxybenzoic and hydroxycinnamic acids (Mekam *et al*., 2019). These phenolics are known bioactive molecules with antifungal properties (Alves Breda *et al*., 2016).

Plant-derived alkaloids were extensively studied for their antifungal properties against human pathogenic fungi, such as *Candida albicans* (Mollataghi *et al*., 2012). Plant alkaloids are also reported to have activity against phytopathogenic fungi. For instance, allosecurinine from *Phyllanthus amarus* (*Euphorbiaceae*) was able to inhibit the growth of *Alternaria* spp. (including *A. solani*), *Fusarium* spp. and other important fungi affecting crop plants (Singh *et al*., 2008). Liu *et al*., (2009) found that sanguinaine, an isoquinoline alkaloid from *Macleaya cordata* (*Papaveraceae*) reduced the growth of *R. solani* at a concentration of 0.45 µg mL^{-1}. Nonetheless, the specific roles of alkaloids extracted from the tested plants remain uncertain, as compared to the possible inhibitory role of phenolics present in the same extracts. This role (if any) does not appear to be important since no significant difference in content was found among the three tested plants.

Regarding polysaccharides, one of the best known with confirmed antifungal effects is laminarin (or laminaran), a glucan with different degrees of molecular branching at β-1,3 and β-1,6. For instance, grey mold (*Botrytis cinerea*) and downy mildew (*Plasmopara viticola*) of grapevine were inhibited by spraying laminarin onto vine canopies (Copping, 2004). Laminarin-based products are commercially available as biopesticides (Environmental Protection Agency, 2010). Activity of laminarin in plant tissues is more related to induced resistance than from direct antifungal effects (Aziz *et al*., 2003). In the present study, polysaccharides were particularly abundant in the WE of *S. cayennensis*, but WE was not more active than EE from this plant. This

suggests that polysaccharides may not have inhibitory effects on fungal growth as great as other compounds in the tested extracts – especially the EEs.

The greater antioxidant capability of a plant extract may be correlated with phenolic composition and concentration. The greater the phenolic content the greater is the antioxidant activity. This is due to the phenolic hydroxyl groups, which stop radical chain reactions via radical scavenging (Shahidi and Chandrasekara, 2010).

Principal component analyses for exploration of relationships between plant extracts and biochemical properties assisted description of variance in the set of multivariate data we obtained (polyphenol, tannin, alkaloid contents and associated fungal growth inhibition). Sample splitting showed in the PC bi-plot clearly reflected the main differences due to extraction method, the plant origins, and the phenolic and alkaloid compositions, which influenced antifungal activity of the different extracts.

Under greenhouse condition, foliar application of EE*eu* on tomato before a challenge pathogen inoculation resulted in increased plant height and the reduction of disease severity caused by *R. solani*. Increased plant height was a beneficial effect of foliar application of EE*eu* during the *in planta* experiments. EE*eu* stimulated plant height more than water in treated tomato plants (Figure 7). This growth promoting activity of EE*eu* was possibly attributable to low molecular weight components, such as plant hormones (gibberellic acids), and to major components such as polysaccharides, amino acids and polyphenols that are involved in many aspects of plant physiology and development. These include seed germination, stem and leaf elongation, flower induction, and fruit and seed development (Kamiya and Garcia-Martinez, 1999). This result is similar to those of Nguefack *et al*. (2013), who reported that spraying rice plants with a 2% ethanol extract, followed by a 2% (w/v) aqueous extract of *Callistemon citrinus* or *Cymbopogum citratus*, increased seedling emergence, tillering, panicles/plant and the grain yields by 25–55%. Zakiah *et al*. (2017) reported that low concentrations of a crude extract of *Cassava asiatica* (25 mg L^{-1}) increased height and leaf area of soybean plants. However, in the present study the tomato-EE*eu*-*R. solani* interaction showed less plant height increase, when compared to the tomato-water interaction. Infection by a root/stem pathogen probably interfered with host metabolism and contributed to reduced plant development.

Management of tomato fungal diseases is primarily achieved through breeding resistant cultivars. Many recently developed cultivars are moderately or very resistant to Fusarium wilt, whereas no tomato variety is currently available that shows acceptable tolerance to damping-off/crown rot. Therefore, we chose *R. solani* as the challenging pathogen for the *in vivo* experiments. Disease reduction reached 29 ± 5% in the tomato-EE*eu*-*R. solani* interaction, in comparison to the tomato-water-*R. solani* interaction, at 20 d after inoculation (Table 1). This suggests that EE*eu* contained natural compounds that possibly act alone or in synergy, stimulating plant defense to provide disease control through induced systemic resistance in tomato against damping-off. This is particularly important since damping-off cannot be easily managed, especially in areas where appropriate crop rotations are not followed. Although extracts from tropical plants are reported to provide crop disease control through stimulation of plant defense systems (Baraka *et al*., 2011; Nashwa and Abo-Elyousr, 2012), *E. hirta* extracts have not been previously examined.

CONCLUSIONS

Natural flora is a source of several biologically active compounds, and some of these have been formulated as botanical agrochemicals and are currently used in the management of agricultural pests (Dubey *et al*., 2011). The present study established that water and hydroethanol extracts obtained from leaves of *O. barrelieri*, *S. cayennensis*, and *E. hirta* are sources of phytochemicals, and these molecules demonstrated inhibitory activity against phytopathogenic fungi. Application of these extracts is, therefore, a promising and environmentally friendly strategy for crop disease control that could contribute to minimizing the risks and hazards of posed by conventional fungicides.

Economic advantages from the use of plant extracts could be particularly relevant in African rural areas, where these pan-tropical plants are common and adapted. Biomolecules may be developed into commercial products by local companies, thus contributing to rural and agro-industrial development, together with increased sustainability for local cropping systems. Current research is devoted to identifying the most effective phenolics and other antifungal compounds (Mekam *et al*., 2019), and compounds showing effective plant growth promotion, to develop and implement innovative plant disease biocontrol strategies.

ACKNOWLEDGMENTS

This research has been supported in part by the European Commission, under the Erasmus+ KA107

mobility project between the University of Modena and Reggio Emilia (Italy) and the University of Yaoundé I (Cameroon).

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