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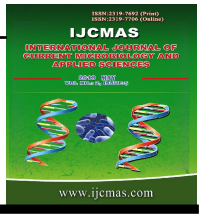

Original Research Article

Antifungal potential and phytochemical analysis of extracts from seven Cameroonian plants against late blight pathogen *Phytophthora infestans*

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Keywords

Late blight; Antifungal; *Phythopthora infestans;* Plants extracts; Phytochemical analysis.

A B S T R A C T

The antifungal potential of extracts from seven Cameroonian plants was evaluated against *Phythopthora infestans*, the devastating Oomycete pathogen of late blight disease of potato and tomato. Essential oils, hot water, cold water and ethanol extracts were obtained from *Ageratum conyzoides, Bidens pilosa, Callistemon citrinus, Cymbopogon citratus, Erigeron floribundus, Ocimum gratissimum* and *Tephrosia vogelii*. The supplemented media technique was used to evaluate the inhibition of the pathogen's mycelial growth by each extracts. Essential oils exhibited the best control of the pathogen, followed by ethanol extracts: total inhibition of pathogen's growth was obtained with essential oils of *C. citratus* at 300 ppm, *O. gratissimum* at 400 ppm, and *C. citrinus* at 5000 ppm. The ethanol extracts of *A. conyzoides* and *C. citrinus* totally inhibited the pathogen at 5000 ppm, and that of *O. gratissimum* at 10000 ppm. The fungitoxic potential of some extracts was comparable to synthetic fungicides used as positive controls. Preliminary phytochemical analysis of water and ethanol extracts revealed that stronger inhibiting effects were recorded with extracts rich in phenolic compounds, sterols, flavonoids, condensed tannins, coumarins and alkaloids. These findings suggest that six extracts obtained from *C. citratus, O. gratissimum, C. citrinus* and *A. conyzoides* possess biofungicidal potential, which can suitably be exploited to control late blight of Solanaceae crops.

Introduction

One of the greatest challenges of world's agriculture today is to improve yield to satisfy the continuous growing food demand, while preserving the environment and human health. Potato (*Solanum tuberosum* L.) and tomato (*Solanum*

lycopersicum L.) are the most important vegetables grown worldwide (FAO, 2011). Many pathogens and pests attack foliage, fruits and tubers of these two Solanaceae crops during growing in field and after harvesting in storage. Late blight caused by the Oomycete *Phytophthora infestans* (Mont.) de Bary is the major disease affecting their global production. (Kapsa and Koodziejczyk, 2005; Foolad *et al.*, 2008). Annual worldwide potato crop losses due to late blight, the disease which was responsible for the Irish potato famine in the 1840s were conservatively estimated at $6.7 billion (Haverkort *et al.*, 2008) thereby making *P. infestans* the single most important biotic threat to global food security. In Cameroon, losses due to late blight have been estimated at 71% in potato and could reach 100% in tomato (Fontem *et al.*, 2005).

Pesticides have been universally used as the most efficient solution to control crop diseases. Sprays of fungicides containing chlorothalonil, metalaxyl, carbendazime, mancozeb and cuprous oxide as active ingredients are commonly used against late blight in Solanaceous crops in Cameroon. However control of late blight by synthetic fungicides creates significant ecological, health and economic issues because of their possible carcinogenicity, high and acute toxicity, long degradation periods, and environmental pollution (Soylu *et al.*, 2006). This increases public demand to minimize the pesticide residues in potatoes and tomatoes products and forces chemical companies and growers to develop and use safer chemical compounds (Strange, 2003). Moreover the resistance developed by plant pathogens has rendered some of them ineffective and increased pathogens aggressiveness. Metalaxyl resistance of *P. infestans* has been recorded in many parts of the world including USA (Lee *et al.*, 1999), Uganda (Mukalazi *et al.*, 2001) and Cameroon (Fontem *et al.*, 2005).

Therefore, late blight appears as a disease to be tackled with alternative products that are environmentally friendly and safe to humans. Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction and growth of plant pathogenic fungi, would be a more realistic and ecologically sound method for integrated plant disease management and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Rhouma *et al.*, 2009). Plant secondary metabolites, contained in extracts of many higher plants are reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory and field tests. Natural products isolated from plant appear to be one of the alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999), as well as large antimicrobial spectra.

In the past, most of the work on antimicrobial effects of essential oils had been conducted on human or food pathogens (Soylu *et al.*, 2006); nevertheless, their application in control of crop plant pathogens is gaining more and more attention. Several studies have reported the effectiveness of essential oils and solvent plant extracts as growth inhibitors of *P. infestans*. Plant extracts from Norway (Quintanilla *et al.*, 2002), from Taiwan (Muto *et al.*, 2005), from

Germany (Krebs *et al.,* 2006), from China (Wang *et al.,* 2001; Cao and Ariena, 2001; Wang *et al.,* 2007) and from Switzerland (Dorn *et al.,* 2007), have shown promising effects against *P. infestans*. Yanar *et al.* (2011), have revealed that out of 26 plant extracts, *Xanthium strumarium, Lauris nobilis, Salvia officinalis* and *Styrax officinalis* were the most active extracts that showed potent antifungal activity on daily radial growth of *P. infestans*. Several Cameroonian plants are reported to possess inhibitory properties against late blight pathogen. Goufo *et al.* (2008), showed that *Cupressus benthamii* and *Vetiveria zizanioides* extracts inhibited sporangial germination and reduced severity of late blight disease on tomato. Methylene chloride/methanol leaf extracts of *Tephrosia vogelli* and *Entandrophragma angolense* significantly inhibited sporangial germination of *P. infestans*, while disease progression was highly limited by *Ageratum houstonianum, Tephrosia vogelli, Clausena anisata* and *Entandrophragma angolense* extracts (Goufo *et al.,* 2010).

Despite the number antimicrobial potential reported from some Cameroonian plants extracts against pathogens, little work on the possible utilization of the essential oils, ethanol and aqueous extracts from the same plants against *P. infestans* has been done. Moreover, the secondary metabolites responsible for their antifungal activities are still to be studied. In this study, 24 plant extracts from seven Cameroonian plants were tested against *P. infestans* under laboratory conditions to determine the effect of these extracts on *in vitro* mycelial growth of the pathogen. Additionally preliminary phytochemical analysis of water and ethanol extracts was performed to determine which groups of secondary metabolites were responsible for the antifungal activity recorded against *P. infestans*.

Materials and Methods

Pathogen's culture

Phytophthora infestans, causal agent of late blight was isolated from ripe tomato fruits obtained from the field and showing symptoms of late blight as described by Fontem *et al.,* (2005) and identified at the Phytopathology Laboratory of the University of Dschang, Cameroon. Cultures of a single isolate of *P. infestans* were maintained on V8 agar medium amended with 50 ppm ampicillin, 50 ppm rifamycin and 0.05 g/L β-sitosterol, in 90 mm diameter Petri dishes at 20 ± 2 °C in darkness and cultures aged 21 days were used for antifungal tests.

Plant material

Seven Cameroonian plants used in this study (Table 1) were selected based on the knowledge of their ethnobotanical uses and their previously demonstrated antimicrobial activities. They were, *Ageratum conyzoides, Bidens pilosa, Callistemon citrinus, Cymbopogon citratus, Erigeron floribundus, Ocimum gratissimum,* collected at Yaoundé (3.8667°N, 11.5167°E) and *Tephrosia vogelii*, harvested at Dschang (5.4500° N, 10.0667°E) during the monsoon period of August 2009. The collected plant parts were air-dried at room temperature (25-27°C) for 10 to 12 days.

Extraction of Essential Oils

The essential oils were extracted from dry plant material by hydrodistillation for five hours using a Clevenger-type apparatus as recommended by Amvam *et al.,* (1998).

Oil collected was dried on anhydrous sodium sulphate (Na_2SO_4) column and preserved at approximately 4°C free from light into airtight brown bottles. The yields of the oils were calculated as percent of plant material weight (% w/w). Essential oils from plants with higher yields (≥ 0.5% w/w) were used for antifungal tests.

Preparation of solvent extracts

Shade-dried plant material of each species was coarsely powdered in a blender and then 100g of powder was first defatted by mixing with 300 mL of hexane for 90 min. After filtration the residue was spread for complete evaporation of the solvent. Lipid-free powder was then soaked and frequently stirred in 500 mL of cold distilled water, or 500 mL of hot water (100°C), or 500 mL of 70% ethanol for 90 min, respectively, followed by filtration first through a double folded cheese cloth, then through Whatman #1 filter paper. The filtrates were subsequently subjected to centrifugation at 7000 rpm for 10 min. Ethanol was totally evaporated from the ethanol extract using a rotary evaporator at 78°C. All supernatants were freeze dried using a lyophilisator and obtained powder of cold water extracts, hot water extracts and ethanol extracts preserved in refrigerator (4°C) into airtight brown bottles until further use.

Synthetic fungicides

Chemicals used in this study included Banko Plus® fungicide titrating 550g/L chlorotalonil and 100 g/L of carbendanzime; Plantizeb® 80WP fungicide containing 80% mancozeb; and Kocide® 2000 titrating 53,8% copper hydroxide. They are among the most used synthetic fungicides by Cameroonian farmers for late blight management.

Antifungal activity test

Inhibitory effect of extracts and synthetic fungicides on mycelial growth of the pathogen grown on V8 agar medium was evaluated using the supplemented media technique as described by Benjilali *et al.*, (1986). Essential oils and synthetic fungicides were added to media at concentrations ranging between 100 and 5000 ppm, while the solvent extracts have been tested at 1000, 5000 and 10000 ppm. Sterile double distilled water was used as negative control. Petri dishes were sealed with parafilm paper and incubated in inverted position at 20 ± 2 °C in darkness for 21 days. The diameter of mycelial growth of pathogen was recorded after 21 days and results expressed as percentage of mycelial growth inhibition (% I) calculated according to the formula of Pandey *et al.*, (1982): % I = (growth diameter in the control – growth diameter in the treatment sample) x 100 / growth diameter in the control.

Determination of the nature of inhibition

Fungal discs from plates in which no colony growth occurred after 21 days of incubation were further checked to detect the fungicidal or fungistatic nature of the inhibition following the procedure of Mishra and Dubey, (1994). The discs were re-inoculated onto the fresh V8 medium and fungal growth was observed during 30 days. The inhibition was qualified as fungistatic if renewed mycelial growth was observed or fungicidal if the contrary was observed.

Preliminary phytochemical screening

Phytochemical tests for major secondary metabolites of the solvent extracts were

performed. Plant extracts were screened for the presence of biologically active compounds such as alkaloids, anthocyanins and cardiac glycosides (Odebiyi and Sofowora, 1978); phenols and flavonoids (Harbone, 1976); triterpenes and sterols, (Schoppe, 1964), saponins (Wall *et al.*, 1952); anthraquinones, hydrolysable and condensed tannins (Trease and Evans, 1989) and coumarins (Kovac-Besović and Durić, 2003). Based on the intensity of coloration or the precipitate formed during the test, secondary metabolites proportion was characterized as strongly present (+++), present (++), weakly present (+) and absent (-) when the test result was negative.

Statistical analysis

Experiments were set in a Completely Randomized Design with three replications. Data were worked out using Statistical Package for Social science (SPSS) version 10.1 software by Analysis of Variance (ANOVA) paired to t-test of Student-Newman-Keuls (parametric) and differences among the means were determined for significance at $P<0.05$.

Results and Discussion

Essential oils characteristics

It appeared that characteristics of essential oils vary from one plant species to another. The highest yield of 1.72% was recorded with *C. Citrinus*. The essential oil yields of *A. conyzoides, B. pilosa, E. floribundus* and *T. vogelii* were very low as compared to *C. citrinus, C. citratus* and *O. gratissimum.*

These three best yielded oil plants were subsequently used for further work. Except essential oils of *A. conyzoides* and *B. Pilosa*, oil colours were generally yellow (Table 1).

Efficacy of essential oils against *P. Infestans*

All tested essential oils exhibited considerable antifungal activity against *P. infestans*, and inhibition of mycelial growth was dose- and plant species-dependant. The essential oils of *C. citratus* and *O. gratissimum* were the most active, with 100% inhibition at 300 ppm and 400 ppm respectively. The essential oil of *C. citrinus* was the less active and total pathogen's inhibition was recorded at 5000 ppm (Table 2).

Efficacy of ethanol extracts against *P. Infestans*

The ethanol extracts of *A. conyzoides* and *C. citrinus*, inhibited significantly ($P<0.05$) the growth of *P. infestans*, with total inhibition at 5000 ppm. The ethanol extract of *O. gratissimum* have totally inhibited the fungal growth at 10000 ppm while 75.29% inhibition was revealed at 5000 ppm. The ethanol extracts of *C. citratus* and *T. vogelii* exhibited similar activities at 10000 ppm with 22.86% growth inhibition whereas at 1000 and 5000 ppm, a stimulatory growth activity of both extracts was observed (Table 3).

Efficacy of water extracts against *P. Infestans*

The mycelial growth of the pathogen was not affected or was lightly inhibited by the majority of cold water extracts whereas considerable growth stimulation was observed with most hot water extracts (Data not shown).

Table. 1 Plants used and their essential oil characteristics.

Plant species	Common name	Family	Plant part	Essential oil colour	Yield (%w/w)
Ageratum conyzoides	Roi des herbes	*Asteraceae*	Whole plant	Pale green	0,12
Bidens pilosa	Hairy Berger	*Asteraceae*	Whole plant	Brown	0,09
Callistemon citrinus	Bottle Brush	*Myrtaceae*	Leaves	Yellow	1,72
Cymbopogon citratus	Fipergrass	*Poaceae*	Leaves	Yellowish	0,68
Erigeron floribundus	Fleabane	*Asteraceae*	Whole plant	Yellow	0,10
Ocimum gratissimum	Massep	*Lamiaceae*	Leaves	Yellowish	0,65
Tephrosia vogelii	Tchieuc	*Papilionaceae*	Leaves	Yellowish	0,13

Table.2 Percentage of mycelial growth inhibition of *P. infestans* obtained with essential oils of *C. citrinus*, *C. citratus* and *O. gratissimum.*

Essential oil concentration (ppm)	Percentage inhibition (%)		
	C. citrinus	*C. citratus*	*O. gratissimum*
100	$0,00^{a}$±0,00	$1,27^{a}$±1,96	$18,42^{a}$±3,01
200	$0,00^{a}$±0,00	$10,13^{b}$±3,73	$23,68^{b}$±9,80
300	$0,00^{a}$±0,00	$100,00^{c}$±0,00	$56,58^{c}$±25,26
400	$0,00^{a}$±0,00	$100,00^{c}$±0,00	$100,00^{d}$±0,00
500	$0,00^{a}$±0,00	$100,00^{c}$±0,00	$100,00^{d}$±0,00
1000	$13,75^{b}$±0,72	$100,00^{c}$±0,00	$100,00^{d}$±0,00
2000	$21,25^{c}$±0,00	$100,00^{c}$±0,00	$100,00^{d}$±0,00
3000	$47,50^{d}$±1,25	$100,00^{c}$±0,00	$100,00^{d}$±0,00
4000	$55,00^{e}$±7,21	$100,00^{c}$±0,00	$100,00^{d}$±0,00
5000	$100,00^{f}$±0,00	$100,00^{c}$±0,00	$100,00^{d}$±0,00

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

Efficacy of synthetic fungicides against *P. infestans*

Synthetic fungicides Banko Plus® and Plantizeb® 80WP completely inhibited the fungus at 100 ppm, and Kocide® 2000 at 5000 ppm.

Nature of the antifungal activity

A renewed growth of the fungus 30 days after transplanting on fresh media was observed on mycelial discs taken from media supplemented with the essential oil and ethanol extract of *C. Citrinus*, and Kocide® 2000 at 5000 ppm. These extracts and this fungicide exhibited a fungistatic effect on the pathogen. Therefore, the concentration of 5000 ppm was recorded as the minimum inhibitory concentration (MIC) of these extracts and synthetic fungicide against *P. infestans*. Transplanting followed by incubation of

mycelial discs taken from the media containing the essential oil of *C. citratus* (300 ppm), *O. gratissimum* (400 ppm), ethanol extract of *A. Conyzoides* (5000 ppm), *O. gratissimum* (5000 ppm) and fungicides fungicides Banko Plus® (100 ppm) and Plantizeb® 80WP (100 ppm) did not led to any fungal growth: they have exerted a fungicidal activity on *P. infestans* and these concentrations were the minimum fungicidal concentrations (MFC) of the extracts and synthetic fungicides (Table 4).

Preliminary phytochemical composition of solvent extracts

Phytochemical screening of solvent extracts showed that their secondary metabolites composition varies with botanical species, method and solvent used for extraction. In general, ethanol extracts contained more secondary metabolites than aqueous extracts. Hot water extracts contained considerable proportions of phenols, flavonoids, cardiac glycosides and condensed tannins, but were all lacking saponins. Cold water extracts mainly contained phenols, triterpenes, cardiac glycosides and condensed tannins but were all deficient in alkaloids and sterols. Phenols were shown to be present in all ethanol extracts and condensed tannins were almost commonly distributed. Also, sterols were weakly detected in five ethanol extracts only. Flavonoids were widespread in different proportions in almost all the extracts. No any extract contained anthocyanins, among ethanol extracts cardiac glycosides were shown only in *C. citratus* and hydrolysable tannins only in *C. citrinus*. Coumarins were identified only in cold water extracts of *C. citratus* and *O. gratissimum*, as well as in ethanol extracts of *A. conyzoides* and *O. gratissimum*. (Table 5)

In this study, the *in vitro* inhibitory effect of 24 extracts from 7 plants on the mycelial growth of *P. infestans* was assessed, and solvent extracts were screened for their secondary metabolites composition. The extraction yields of essential oils varied with plant species. Lower yields were obtained with *B. pilosa* (0.09%), *E. floribundus* (0.10%), *A. conyzoides* (0.12%) and *T. vogelii* (0.13%). Higher yields of 0.65%, 0.68% and 1.72% were obtained with *O. gratissimum, C. citratus* and *C. citrinus* respectively. This yield of *C. citratus* is different from 0.57% yield obtained by Nguefack *et al.*, in 2005. Bengyella *et al.*, (2011) have obtained 1.46% oil yield from *O. Gratissimum*. These disparities confirm the hypothesis that essential oil extraction

Table.3 Percentage of mycelial growth inhibition of *P. infestans* obtained with ethanol extracts of the seven plants

Plant species	Percentage inhibition (%)		
	Ethanol extract concentration (ppm)		
	1000	5000	10 000
Ageratum conyzoides	46,66^{a}± 0,67	100,00^{b} ±0,00	100,00^{b} ±0,00
Bidens pilosa	0,00^{a} ±2,48	2,86^{b} ±2,48	64,29^{c} ±0,00
Callistemon citrinus	0,00^{a} ±2,67	100,00^{b} ±0,00	100,00^{b} ±0,00
Cymbopogon citratus	-7,14^{a} ±1,43	-10,00^{b} ±0,00	22,86^{c} ±3,78
Erigeron floribundus	6,25^{a} ±5,28	12,50^{b} ±4,92	11,25^{c} ±12,90
Ocimum gratissimum	18,82^{a} ±0,00	75,29^{b} ±13,93	100,00^{c} ±0,00
Tephrosia vogelii	-4,29^{a} ±0,00	-7,14^{b} ±0,00	22,86^{c} ±1,43

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

Table.4 Nature of inhibition of *P. infestans* mycelial growth by plant extracts and synthetic fungicides.

Plant extracts and fungicides	Inhibitory Concentration (ppm)	Antifungal property
Essential oil *C. citrinus*	5000	Fungistatic
Essential oil *C. citratus*	300	Fungicidal
Essential oil *O.gratissimum*	400	Fungicidal
Ethanol extract *A. conyzoides*	5000	Fungicidal
Ethanol extract *C. citrinus*	5000	Fungistatic
Ethanol extract *O.gratissimum*	10000	Fungicidal
Kocide® 2000	5000	Fungistatic
Plantizeb® 80WP	100	Fungicidal
Banko Plus®	100	Fungicidal

yield could be influenced by intrinsic factors such as botanical species and plant vegetative cycle; and extrinsic factors such as climatic conditions, soil type, place and time of harvest (Bruneton, 1999).

In the present study, the essential oils showed the highest antifungal activity against *P. infestans* as compared to ethanol and aqueous extracts of the same plant. Similar observations have been reported by other authors. Mihailović *et al.,* (2011) observed that antimicrobial activity of *Gentiana asclepiadea* essential oil (MIC values: 0.62- 2.5 µl/mL) was higher than the ones of methanolic and n-butanolic extracts (MIC values: 312.5 to 2500 µg/ml) of the same plant. Bengyella *et al.,* (2011) reported that *O. gratissimum* essential oil at 150 ppm inhibited by 86.17 and 100% the mycelial growth of *Bipolaris oryzae* and *Alternaria padwickii* respectively. The ethanol extract at 10000 ppm showed 80.92 and 61.54% growth inhibition of *B. oryzae* and *A. padwickii* respectively. *Cymbopogon citratus* oil was the most active with MFC of 300 ppm followed by *O. gratissimum* with MFC of 400 ppm and *C. citrinus* with 5000 ppm as MIC. Effectiveness of essential oils on *P. infestans* has been previously reported. Quintanilla *et al.,* (2002) observed that four essential oils i.e. thyme, oregano, lemon balm and peppermint moderately inhibited *P. infestans* mycelial growth (63-89% inhibition). Various essential oils demonstrated significant inhibition at 100 and 1000 ppm, over 90% inhibition of *P. infestans* growth was obtained with oregano and Serenade amendments (Olanya and Larkin, 2006). Soylu *et al.,* (2006) shown that oregano, thyme and fennel oils at 6.4 µg/mL (6.4 ppm) completely inhibited mycelial growth of *P. infestans* whereas growth was totally inhibited by rosemary and lavender essential oils at 12.8 and 25.6 µg/mL (12.8 and 25.6 ppm) concentrations respectively.

According to Mishra and Dubey (1994) and Amvam *et al.,* (1998), antimicrobial activity of an essential oil is related to its chemical composition. In addition, the activity of an essential oil is much related to its proportion in oxygenated terpenes (Hammer *et al.,* 2003; Nguefack *et al.,* 2012). It is therefore evident that *C. citratus* oil which contains 90.4% oxygenated terpenes (Nguefack *et al.,* 2007) was most active. Well-known active ingredients in the chemical composition of essential oils could justify their high antifungal activities against *P. infestans*.

Table.5 Preliminary phytochemical analysis of cold water, hot water and ethanol extracts.

Secondary metabolites	Solvent extract	*A. conyzoides*	*B. pilosa*	*C. citrinus*	*C. citratus*	*E. floribundus*	*O. gratissimum*	*T. vogelii*
Alkaloids	ETE	+	++	-	-	+	-	-
	CWE	-	-	-	-	-	-	-
	HWE	-	++	+	-	-	++	-
Phenols	ETE	+++	+++	+++	++	++	++	+++
	CWE	+	+	-	-	-	+	+
	HWE	+	+	+	+	-	+	-
Triterpenes	ETE	-	-	+	-	-	-	+
	CWE	+	++	-	-	+	++	-
	HWE	+	-	-	-	+	+++	-
Sterols	ETE	+	+	+	-	+	+	-
	CWE	-	-	-	-	-	-	-
	HWE	-	-	-	-	-	-	-
Flavonoids	ETE	+	++	+++	+	+	+	+++
	CWE	+	+	-	-	+	+	+
	HWE	+	+	+	+	+	+	+
Saponins	ETE	-	-	+	+	-	-	+
	CWE	-	-	+	-	-	-	-
	HWE	-	-	-	-	-	-	-
Anthocyanins	ETE	-	-	-	-	-	-	-
	CWE	-	-	-	-	-	-	-
	HWE	-	-	-	-	-	-	-
Anthraquinones	ETE	-	++	++	+	-	-	+
	CWE	-	+	+	-	-	-	-
	HWE	-	+	+	-	-	-	+
Cardiac glycosides	ETE	-	-	-	+	-	-	-
	CWE	+	+	-	+	+	+	+
	HWE	++	++	-	++	+	++	++
Coumarins	ETE	+	-	-	-	-	+	-
	CWE	-	-	-	+	-	+	-
	HWE	-	-	-	-	-	-	-
Hydrolysable tannins	ETE	-	-	+++	-	-	-	-
	CWE	-	-	-	-	-	-	-
	HWE	-	-	-	-	-	-	-
Condensed tannins	ETE	++	+++	-	++	+	++	+++
	CWE	+	++	++	-	+	+	++
	HWE	++	+++	+++	+	+	++	++

Strongly Present: +++; Present: ++; Weakly Present: +; Absent: -
Ethanol extract: ETE - Cold water extract: CWE - Hot water extract: HWE

Thus, the strong activity of *C. citratus* might be due to its proportion in neral and geranial, which represent the major constituents (84.21% of total oil composition). Similarly, activity of *O. gratissimum* oil could be linked to thymol, γ-terpinene and p-cymene (73.20%) (Nguefack *et al.*, 2007), whereas 1,8-cineole, α-pinene and α-terpineol (94.90%) could be responsible for *C. citrinus* activity (Jazet *et al.*, 2009; Dongmo *et al.*, 2010). The activity of these terpenes results from their high solubility in aqueous media and in microbial membranes (Dorman and Deans, 2000; Hammer *et al.*, 2003). Essential oil active compounds inhibit *P. infestans* by provoking considerable morphological alterations in fungi hyphae such as cytoplasmic coagulation, vacuolations, hyphal shrivelling and protoplast leakage (Soylu *et al.*, 2006).

The solvent extracts revealed diverse antifungal activities, depending on whether one considers the type of extract or plant species. Considering the type of extract, in general, ethanol extracts were most active followed by cold water extracts. These results are in agreement with previous studies showing the antifungal activity of *O. gratissimum* extracts against *B. oryzae* (Bengyella *et al.*, 2011). Ethanol extracts from *A. conyzoides* (MFC=5000 ppm), *C. citrinus* (MIC=5000 ppm) and *O. gratissimum* (MFC=10000 ppm) exhibited the most interesting results. Moreover, *B. pilosa* ethanol extract shown 64.29% inhibition at 10000 ppm. These results are similar to those obtained by Wang *et al.*, (2001), Cao and Ariena (2001) and Wang *et al.*, (2004), with different plant extracts against *P. infestans*. Several other studies reported that plant solvent extracts play an important role in controlling the late blight pathogen. Methylene chloride and methanol (1:1 V/V) extracts of *Cupressus benthamii* and *Vetiveria zizanioides* at 3% (30000 ppm) shown 23% and 35% inhibition of sporangial germination of *P. infestans* respectively (Goufo *et al.*, 2008). Cold water extract of six medicinal plant species i.e. *Amaranthus spinosus, Barbeya oleoides, Clutia lanceolata, Lavandula pubescens, Maerua oblongifolia* and *Withania somnifera* in total reduced by 29.6% the mycelial growth of *P. infestans* and spore germination was inhibited by 16–65%, 18–75%, and 21–79% at concentrations of 2.5%, 5% and 10%, (25000, 50000 and 100000 ppm) respectively (Baka, 2010). Yanar *et al.*, 2011 demonstrated that out of 26 plant extracts, *Xanthium strumarium, Lauris nobilis, Salvia officinalis* and *Styrax officinalis* were most active on daily radial growth of *P. infestans* and completely inhibited mycelial growth of the pathogen at 4% (40000 ppm) concentration. Abd-El-Khair and Wafaa (2007) observed that cold water extracts of basil leaves (*Ocinum bacilicum*), chilli fruits (*Capsicum frutescens*), eucalyptus leaves *(Eucalyptus globulus*), garlic bulbs (*Allium sativum*), lemon grass leaves (*Cymbopogon citratus*), marjoram leaves (*Majorana hortensis*), onion seeds (*Allium cepa*) and peppermint leaves (*Mentha piperita*) reduced the spores germination (%) of *P. infestans* from 30 to 56%, 41 to 72%, and 58 to 81% at concentrations of 2.5%, 5.0 and 10.0%, respectively.

The highly inhibition of mycelial growth of *P. infestans* was obtained with lemon grass leaves followed by garlic bulbs, onion seeds, basil leaves, eucalyptus leaves, peppermint leaves, marjoram leaves, chili fruits and lantana leaves & fruits, respectively. They gave the values of mycelial growth reduction (%) 68.9,

61.0, 56.1, 55.2, 53.6, 48.3, 47.0, 46.9 and 46.2%, respectively.

A tentative correlation between antifungal activity and phytochemical composition of solvent extracts suggests that ethanol extracts with significant antifungal activity mainly contain phenols, sterols, flavonoids, condensed tannins, and to a lesser extent coumarins and alkaloids. This suggests a high solubility of these secondary metabolites in ethanol, or the absence of their inhibitors in ethanol extracts (Quasem and Abu-Blan, 1996, Lapornik *et al.,* 2005). Phenols at high concentration have been reported to have very high antimicrobial activity. Also, high activity of coumarins such as phytoalexins produced by plants in response to fungal attack has been reported by many authors (Cowan, 1999; Lapornik *et al.,* 2005). This could explain the fact that three extracts containing coumarins (*A. conyzoides* and *O. gratissimum* ethanol extracts; and *C. citratus* cold water extract) were very active against *P. infestans*. Hot water extracts did not show any inhibitory effect on the pathogen. This could result from destruction of some thermally labile antifungal compounds by heat during extraction (Quasem and Abu-Blan, 1996; Lapornik *et al.,* 2005).

Stimulation of fungal growth by several extracts was observed in this work. Several reports mentioned stimulation of pathogens growth by plant extracts (Wang *et al.,* 2001; Bengyella *et al.,* 2011). This could be explained by low phenols content of the extracts as revealed by phytochemical analysis. In facts, low concentrations of phenol (3-5 µg/mL) are required by fungi during normal metabolism but higher concentrations (20 µg/mL) are inhibitory to fungal growth (Mohapotra *et al.,* 2000). Furthermore, the lesser activity of cold water extracts could be due to their lesser concentrations in active secondary metabolites as shown from phytochemical screening, because of the low water solubility of these metabolites. It could also be due to the water extraction of polysaccharides, amino acids and proteins that could reduce the quantity and the activity of active metabolites. Actually, thin layer chromatography of *C. citrinus* water extracts revealed the presence of arginine and lysine. The presence of these primary metabolites can explain also the stimulatory growth effect observed with some cold and hot water extracts (Nguefack, Personal communication).

Considering the different plant species, it appeared that the composition of secondary metabolites varies from one species to another. Javed *et al.,* 2011 also reported a diversified phytochemical composition of essential oil, aqueous, methanol and chloroform extract of *Eucalyptus citriodora* leaves. Methanol extracts were most rich in secondary metabolites and considerable proportions of sterols and phenols were predominantly found in all four extracts. The presence of active compounds in a sample is influenced by the extraction method, age of the plant, harvest time and the extraction solvent (Lapornik *et al.,* 2005; Tiwari *et al.,* 2011).

In conclusion, this work has revealed exploitable potential of *Callistemon citrinus*, *Cymbopogon citratus* and *Ocimum gratissimum* essential oils, as well as *A. conyzoides*, *C. citrinus*, and *O. gratissimum* ethanol extracts as prospective source of compounds effective against *P. infestans*. The degree of fungal growth inhibition by these extracts

recorded here for the first time was stronger than many other reported antifungal plant extracts against *P. infestans*. This study has also revealed the presence of bioactive groups (phenolic compounds, sterols, flavonoids, condensed tannins, coumarins and alkaloids) in plant extracts. These findings represent important first steps towards isolation and characterization of antifungal agents and their further *in vivo* utilisation in crop protection strategies.

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