Current Topics in Phytochemistry
Vol. 16, 2020

Original Article

Antifungal potential of essential oil from *Zanthoxylum pseudodumosum* Beurton, an endemic species of Cuba

Lisbet Martín Cruz[1], Pierre Betu Kasangana[2], Katia Ojito-Ramos[3], Orelvis Portal[3,4], José O. Guerra de León[1] and Tatjana Stevanovic[2,*]

[1]Departamento de Licenciatura en Química, Facultad de Química y Farmacia, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba. [2]Centre de Recherche sur les Matériaux Renouvelables, Université Laval, Québec, QC, Canada. [3]Departamento de Biología, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba; [4]Centro de Investigaciones Agropecuarias, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba.

ABSTRACT

For the first time, the essential oil from leaves of *Zanthoxylum pseudodumosum*, an endemic species from central Cuba, was analysed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). A total of seventeen compounds representing 85.7% of the oil were identified and the major components were revealed to be mainly sesquiterpene hydrocarbons, among which the major were β-caryophyllene, (32.01%), germacrene D (14.89%) and α-selinene (8.67%). Antifungal activity and the capacity of the essential oil to induce oxidative stress were evaluated against the phytopathogenic fungus *Alternaria solani*. The results showed that the oil exhibited an inhibitory effect on mycelial growth of *A. solani* and caused oxidative stress by increasing the concentration of malondialdehyde (MDA) in comparison with the control. These findings therefore confirm the antifungal effect of *Z. pseudodumosum* leaf essential oil and the potential uses of its use as a natural product, an alternative to chemical fungicides.

KEYWORDS: *Zanthoxylum pseudodumosum*, essential oil, chemical composition, antioxidant activity, antifungal activity.

*Corresponding author: Tatjana.Stevanovic@sbf.ulaval.ca

INTRODUCTION

The genus *Zanthoxylum* (Rutaceae) comprises many species distributed worldwide mainly in tropical and temperate regions; it is the only genus of this family that has a natural pantropical distribution [1]. The essential oils derived from the genus *Zanthoxylum* were reported to have a wide variety of biological activities such as antimicrobial, antioxidant, anti-inflammatory as well as citotoxic properties [2]. In Cuba, there are twenty-five *Zanthoxylum* species, of which thirteen are endemic. Phytochemical studies conducted so far on the species from the *Zanthoxylum* genus growing in Cuba, reported the presence of alkaloids, coumarins, tannins, phenols, saponins, terpenoids and steroids [3], but the essential oil' composition for these endemic species is practically unknown.

Z. pseudodumosum Beurton is an endemic shrub in central Cuba, which grows on spiny xeromorph brushwood on serpentine soils [3]. There are no reports about its ethnobotanical uses in the communities where this species grows or about its chemical composition. The leaves' extracts of *Z. pseudodumosum* have been previously evaluated in *in vitro* conditions against *Alternaria solani* Sor. phytopathogenic fungus of the potato and tomato [4]. The "early blight", caused by this fungus, is one of the most destructive fungal diseases

that reduces the quality, quantity and market value of potato tubers [5]. This fungus also causes several diseases in tomato and is considered one of the most harmful under Cuban agroecological conditions [6]. The control of the mentioned diseases is mainly done through the use of chemical fungicides, which make production more expensive, while their use is also harmful to the environment [6]. At present, the development of products from plants is a promising alternative, since efficiency effect of the extracts against the development of phytopathogenic fungi has been demonstrated [7].

Therefore, the aim of this study was to investigate, for the first time, the chemical composition of essential oil extracted from leaves of endemic species *Z. pseudodumosum*, and also to evaluate its antifungal potential against *A. solani* with a possible action mode.

MATERIALS AND METHODS

Plant material and essential oil isolation

The leaves of *Z. pseudodumosum* were collected in their natural habitat in Camajuaní road, Santa Clara, in the central region of Cuba (LAT 22.45199; LONG 79.8161) in September 2018. The plants were identified by PhD. Idelfonso Castañeda Noa from the Botanic Garden at Universidad Central "Marta Abreu" de Las Villas. A voucher specimen was stored in the herbarium of this institution (No. 12414 ULV). The leaves were dried in an air-circulating oven under 40 ºC, then were crushed and stored to preserve them from moisture and other contaminants.

Dried leaves (80 g) were subject to hydro-distillation for 3 h using a modified Clevenger-type apparatus with 4 L of distilled water. The essential oil was separated from the distillate by liquid-liquid extraction with 30 mL of hexane (in triplicate), dried over anhydrous sodium sulfate and stored in sealed vials at -4°C. This procedure was performed in triplicate to provide samples for determination of essential oil's composition and antifungal activity.

Gas chromatography analysis of essential oil

Qualitative analyses were carried out on a Varian Saturn 2200 GC/MS/MS equipped with an Agilent J & W VF-5ms with a 5% phenyl-methyl column (30 m x 0.25 mm x 0.25 µm film thickness). One microliter of sample diluted in hexane was injected with a split ratio 1:10. Operating conditions were as follows: oven temperature program from 50 ºC-200 ºC at rate of 3 ºC/min; injector and detector temperatures were 250 ºC and 285 ºC respectively; carrier gas He at 1 mL/min. The mass spectrometer was operated in electronic ionization mode at 70 eV and the signal acquisition was set between 30-600 m/z. The gas chromatography-flame ionization detector (GC/FID) analysis was carried out on Varian CP-3800 gas chromatography (Varian Inc., Walnut Creek, USA), equipped with a flame ionization detector (FID). The column and conditions of temperature were identical to those used in the gas chromatography-mass spectrometry (GC-MS) analysis.

Identification of the essential oil constituents was performed by comparison of the mass spectra with those available in the NIST 02, Adams and Essentia database, provided by a computer controlled GC-MS system, and with mass spectra literature [8]. The retention indices were calculated for all the volatile constituents using the retention data of linear n-alkanes C7-C30.

Mycelium growth inhibition

The *A. solani* isolate was obtained from the microbial culture collection of the Instituto de Biotecnología de las Plantas, Santa Clara, Cuba. *Z. pseudodumosum* essential oil was tested in triplicate for each assay and repeated once.

For the minimal inhibitory concentration (MIC) determination, the *Z. pseudodumosum* essential oil, dissolved in 2.5% dimethylsulfoxide (DMSO), was tested at four concentrations (4, 2, 1 and 0.5 mg/mL) in 100 µL of potato dextrose broth (PDB) culture medium in 96 wells microliter plates (Eppendorf, Germany). Then, 100 µL of an *A. solani* suspension (5 × 105 mycelial fragment/mL) was added to each well. Tebuconazole (1 mg/mL, Orius 25 EW) (INICA, Venezuela) and 40% DMSO were used as positive controls, while *A. solani* in PDB culture medium and *A. solani* in PDB culture medium with 2.5% DMSO were used as negative controls. The plates were incubated at 27 °C for 72 h, at 100% relative humidity in darkness and the MIC was defined as lowest concentration of *Z. pseudodumosum* essential oil, which inhibited *A. solani* fungal growth.

The effect of *Z. pseudodumosum* essential oil on the radial mycelial growth of *A. solani* was measured using the Agar dilution method. Disks of 0.6 cm diameter from a seven days-old culture of *A. solani* were placed in the center of Petri dishes (9 cm diameter) containing potato dextrose agar culture medium with the *Z. pseudodumosum* essential oil at a concentration according to MIC results. Then, petri dishes were incubated at 27 °C in darkness. Tebuconazole 1 mg/mL and 40% DMSO were used as positive (antifungal) controls, and *A. solani* in PDB culture medium and *A. solani* in PDB culture medium with 2.5% DMSO were used as negative (growth) controls.

The diameter of *A. solani* mycelial growth was measured daily during seven days. The percentage of mycelial growth inhibition was calculated using the formula [(C-T)/C*100], where C and T correspond to the hyphal length (cm) in the control and treated fungal cultures, respectively.

Oxidative stress

The oxidative stress caused by the *Z. pseudodumosum* essential oil was determined by the activity of the antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). Besides, the biomolecule damage was determined by membrane lipoperoxidation and advanced oxidation protein product (AOPP) determination. Each determination was made in triplicate and the experiments were repeated once.

A. solani mycelium (0.5 g) grown under the conditions described above were crushed in 50 mL of 5 mM Tris-HCL buffer (pH 7.0), to which the *Z. pseudodumosum* essential oil was added (according to the MIC results) and incubated at 27 °C for 48 h. As a control culture (essential oil not treated), the fungus was mixed in 5 mM Tris-HCL buffer (pH 7.0). Three replications per treatment and control were performed. Subsequently, the *A. solani* mycelium grown in the above conditions was centrifuged at 5000 x g for 10 min. After removing the supernatant, the pellet was washed with distilled water and centrifuged at 8000 x g for 10 min. Next, the supernatant was removed and the pellet was resuspended in 3 mL of cold lysis buffer (50 mM potassium phosphate buffer (pH 7.0), 1 N NaCl, 1% polyvinylpyrrolidone MW 40000, 1 mM ascorbate) and centrifuged at 15000 x g for 20 min at 4 °C. The supernatant was used for the determination of the activity of antioxidant enzymes and advanced protein oxidation products and the pellet were used for the determination of lipid peroxidation. The results were standardized relative to the total protein content.

The SOD activity was determined by measuring the inhibition of the photochemical reduction of the blue tetrazolium reagent (NBT) [9]. In addition, the APX activity was determined by measuring the decomposition or degradation of ascorbate at 290 nm for 45 s [10]. Furthermore, the CAT activity was determined by the decrease in absorbance at 240 nm [9].

The membrane lipoperoxidation was determined by the thiobarbituric acid (TBA) method, which is based on the production of malondialdehyde (MDA) [11]. Besides, the AOPP was determined according to Matteuci *et al.* [12].

Estimation of antioxidant activity using DPPH free radical scavenging method

Equal volumes of methanolic solution of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (200 μM) and of the methanolic solution of essential oil were mixed in a Rapid Kinetic Accessory SF-22 (Hi-Tech Scientific, Salisbury, UK) coupled with Cary UV Spectrophotometer. The temperature was kept at 25 °C. The radical scavenging effect was continuously followed by monitoring the change of absorbance at 516 nm for 30 min, against a methanol solvent blank. The ascorbic acid and Oligopin® (1 mg/mL), a standardized maritime pine bark extract (DRT Nutraceutics, France), were used as the positive control.

Statistical analysis

The results are expressed as the mean ± standard error of triplicated data, according to the Mann Whitney U analysis ($p < 0.05$). Descriptive statistical analysis was performed using IBM SPSS Statistics, version 21 for Windows.

RESULTS AND DISCUSSION

Chemical composition of essential oil

As mentioned in the introduction, there is a lack of rigorous information concerning the chemical

composition and biological effects of the essential oil from Cuban *Z. pseudodumosum*'s leaves, notably in terms of its traditional use against phytopathogenic fungi of the potatoes and tomatoes. In this study, the leaf essential oils of *Z. pseudodumosum*, yellow in color and with a characteristic odor, were obtained by hydro-distillation. The average extraction yield was determined to be 0.43% ± 0.02 (w/w), which is lower than the yields reported for essential oils from other *Zanthoxylum* species, which were between 1.78 and 4% [13].

The chemical composition of the essential oil was analyzed by GC-FID and GC-MS (Figure 1). As shown in Table 1, seventeen compounds, representing 85.7% of the total oil, were identified. The sesquiterpenes (oxygenated and hydrocarbons) make up the highest percentage (80.47%) of the components identified in *Z. pseudodumosum* leaf oil whilst the monoterpenes represent only 0.44%. The composition of *Z. pseudodumosum* leaf essential oil is thus in contrast with the composition of the majority of essential oils from other Z*anthoxylum* species which are often complex mixtures of monoterpenes and sesquiterpenes.

The major constituents of the essential oil from *Z. pseudodumosum* leaves were determined to be (*E*)-Caryophyllene, (32.01%) followed by Germacrene D (14.89%) and α-Selinene (8.67%). These results slightly differ from previous reports on essential oils from other *Zanthoxylum* species, in which only the sesquiterpene hydrocarbon, germacrene D has been reported as one of the main constituents, notably in *Z. rhoifolium* oil and *Z. fagara* oil [14, 15]. Another study indicated that sabinene (25.71%), 1,8-cineole (9.19%), and cis-4-thujanol (9.19%) were the major constituents of *Z. monophyllum* oil whereas the pericarp oil of *Z. bungeanum* mainly contained D-limonene (22.19%), β-myrcene (9.66%), trans-β-ocimene (9.58%), terpinen-4-ol (8.96%), and γ-terpinene (4.45%) [16, 17].

For the essential oil obtained through hydrodistillation of *Z. caribaeum* leaves, 60.92% was sesquiterpenes and 2.96% monoterpenes, representing a total of 63.88%; the majority compounds being Germacrene D (20.77%), α-Panasinsene (14.40%) and β-Selinene (11.68%) [18]. The essential oil of the leaves of *Z. ekmanii*, from the southern Amazon forest, Brazil, showed Germacrene D (16.0%) and β-caryophyllene (11.5%) as the major components [19].

Additionally, the presence of mint sulfide is noteworthy in leaf essential oil of *Z. pseudodumosum*, representing 4.74% of total oil.

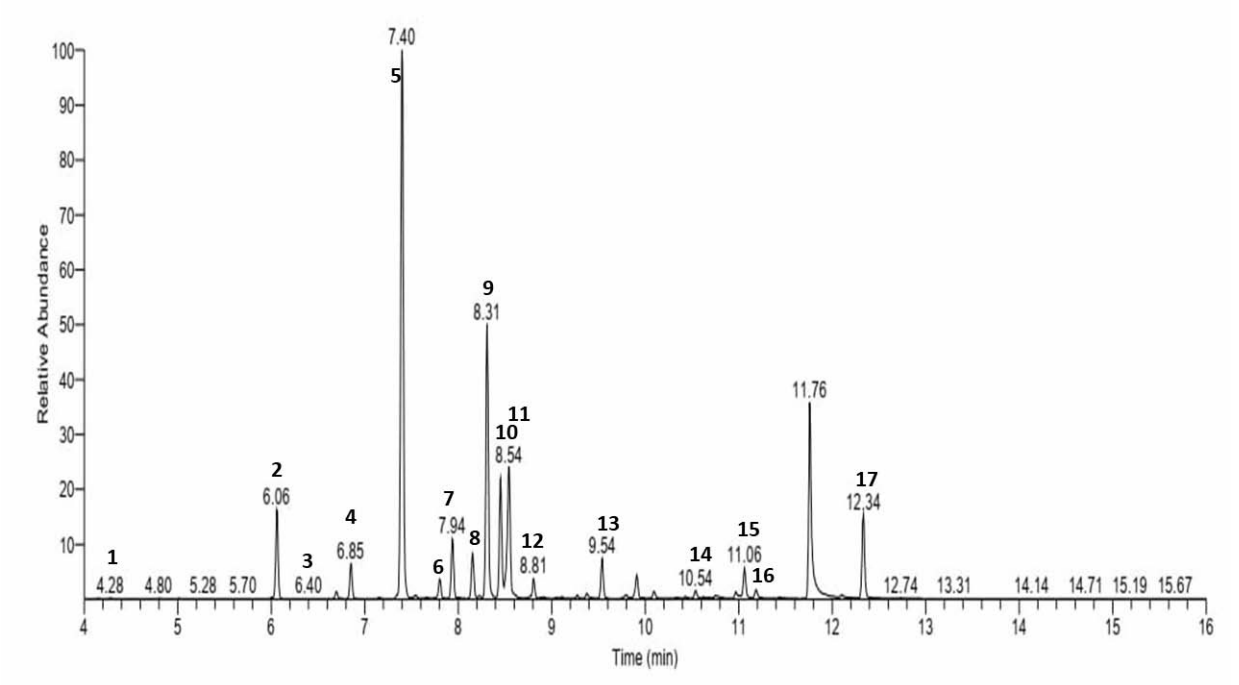

Figure 1. GC-FID profile of essential oil of *Z. pseudodumosum*; identified peaks are numbered.

Table 1. Composition (%) of essential oil of *Z. pseudodumosum.*

Peak n°	Compounds	[a]RI	[b]%	[c]Identification Methods
1	Camphene	946	0.44	[c]MS, RI
2	δ-Elemene	1336	4.66	MS, RI
3	α-Cubebene	1387	0.46	MS, RI
4	β - Elemene	1389	1.86	MS, RI
5	**(E)-Caryophyllene**	1418	**32.01**	MS, RI, Std
6	α - Guaiene	1436	1.14	MS, RI
7	Aromadendrene	1439	3.16	MS, RI
8	γ-Humulene	1452	2.53	MS, RI
9	**Germacrene D**	1483	**14.89**	MS, RI, Std
10	**β-Selinene**	1492	**6.43**	MS, RI
11	**α-Selinene**	1494	**8.67**	MS, RI
12	γ - Cadinene	1510	1.12	MS, RI
13	δ - Cadinene	1525	2.15	MS, RI
14	Spathulenol	1574	0.49	MS, RI
15	τ -Cadinol	1636	0.39	MS, RI
16	Eudesmol	1653	0.51	MS, RI
17	Mint sulfide	1738	4.79	MS, RI
	Percentage of total identification		**85.70**	
	Monoterpene hydrocarbons		**0.44**	
	Sesquiterpene hydrocarbons		**79.08**	
	Oxygenated sesquiterpenes		**1.39**	
	Others		**4.79**	
	Unidentified compounds		**14.30**	

[a]RI: Retention indices taken from Adams.
[b]% Relative area percentage of the compounds obtained from FID area percent data.
[c]Identification methods: MS, by comparison of the mass spectra with those from computer mass libraries, NIST 02 library, Adams and Essentia databases; Std, by comparison of the retention time and mass spectra of authentic standards.
The major compounds are indicated in bold.

The presence of sulfur-containing sesquiterpene is often responsible for the characteristic and strong odor of the oil. Mint sulfide occurs particularly in the essential oil of peppermint, in which this compound was an undesirable component. In fact, mint sulfide was reported to result from a conversion of germacrene D by a photochemical reaction in the presence of elemental S [20, 21].

The chemical composition of the essential oils from *Zanthoxylum* species seems to vary in a significant manner in terms of sesquiterpene and monoterpene compositions [16, 17]. This variability could be attributed to the large genetic and species diversity of the genus *Zanthoxylum*, and also to the differences in the tissues (leaf or whole plant etc.) from which the essential oils were extracted

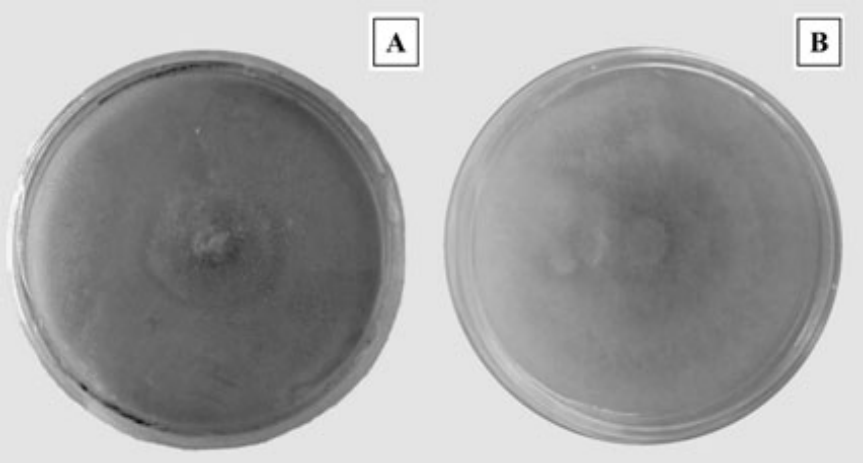

Figure 2. *Alternaria solani* grown on potato dextrose agar culture medium with essential oil from *Z. pseudodumosum* at 2 mg/mL (**A**) and without (**B**) essential oil.

as well as the influence of diverse growth conditions, harvest time and extraction techniques applied.

Mycelium growth inhibition

The essential oil of *Z. pseudodumosum* leaves showed antifungal activity at concentrations above 2 mg/mL, which correspond to the MIC value. At this concentration, the essential oil inhibited the mycelial growth of *A. solan*i by 61% (Figure 2), which open new perspectives for the control of this pathogen. According to the revised literature, this result represents the first study where the antifungal activity of essential oils of *Z. pseudodumosum* is informed.

The negative impact of *A. solani* on the economy has incited the interest for searching for alternative and sustainable solutions in controlling this pest, such as the application of essential oils [6]. In this sense, essential oils from *Carum copticum*, *Oliveria decumbens*, *Thymus kotschyanus*, *Cinnamomum zeylanicuma* at 1.0 µL/mL [22] and *Laurus nobilis* at 10 µL/mL [23] showed a maximum inhibitory effect (100% inhibition). In addition, the essential oil from *Lippia alba* at 0.1 µL/mL was able to control the mycelial growth of *A. solani* by 50% [24].

The comparison of these results with those obtained in this work is difficult due to the use of different concentration units. On the other hand, essential oils from *Eucalyptus gomphocephala* showed 50% of mycelial growth inhibition at 0.99 mg/mL [25]. This concentration of the essential oil is slightly lower than those used in this work (2 mg/mL), where 61% inhibition was obtained, which can be considered as a potent [26] and promising activity [27]. This result is of great importance for control of early blight in tomatoes and potatoes. Major compounds identified in the essential oil of *Z. pseudodumosum* have been identified in the essential oil of *L. alba* with antifungal activity against *A. solani* (β-caryophyllene 2.69% and Germacrene D 3.33%) [24].

The antifungal activity of essential oils is particularly related to the lipophilic nature of their constituents, which explains their affinity for the cell membranes and could constitute the main mode of action [28]. Therefore, the essential oil from leaves of *Z. pseudodumosum*, composed primarily of hydrocarbon sesquiterpenes, should by its nature have an affinity for the *A. solani* cell walls.

Oxidative stress

The induction of oxidative stress is characterized by high levels of free radicals and an impaired antioxidant defense system. During this process, antioxidant enzymes are involved in maintaining the balance between the production and removal of H_2O_2, among which are SOD, CAT and APX [9].

There were no significant differences in the SOD and APX activity of *A. solani* treated with the essential oil of *Z. pseudodumosum* with respect to

the control culture (essential oil-not treated) after 24 h of incubation. However, the CAT activity was lower after 24 h of incubation with the essential oil (Figure 3). CAT plays a fundamental role in the protective capacity of biological systems against reactive oxygen species (ROS); the inhibition of this enzyme causes the accumulation of free radicals, with putative cellular damage [9]. It has been shown that some sesquiterpene lactones such as parthenolide and its water-soluble form dimethylamino parthenolide decrease CAT activity [29, 30].

The modification of the activity of antioxidant enzymes is not enough to confirm the existence of oxidative stress, as there must be evidence of damage to biomolecules such as lipids, proteins and nucleic acids. The oxidation of lipids is one of the evidences of oxidative stress induced by ROS. They react rapidly with unsaturated lipids and produce polar lipid hydroperoxides, which can cause an increase in membrane fluidity due to the breakdown of hydrophobic phospholipids. The final products of this oxidation, such as MDA, are used as a marker of oxidative damage [31].

The MDA concentration in *A. solani* increased after 24 h of incubation with the essential oil, compared to the control culture (essential oil-not treated) (Figure 4A), which indicates the lipid peroxidation of the membrane of *A. solani* and thereby oxidative damage. However, there were no significant differences in AOPP concentration with respect to control culture (Figure 4B).

The induction of oxidative stress in *A. solani* could be due to the action of sesquiterpenes, the major compounds in the essential oil of *Z. pseudodumosum*. Sesquiterpenes are natural compounds with 15-carbons in their skeleton. These compounds can be hydrocarbon or oxygen-based and contain alcohol, ketone, aldehyde, acid, and the lactone groups, which gives them a variety of biological activities *e.g.* induction of oxidative stress [29, 32, 33].

Estimation of antioxidant activity using DPPH free radical scavenging method

The DPPH assay measures the radical scavenging activity of the tested substances. A freshly prepared DPPH solution exhibits a deep purple color that generally fades or disappears when an antioxidant (radical scavenger) is present in the medium. Thus, antioxidant molecule can react with DPPH free radicals and convert them into a colorless product resulting in a decreasing absorbance.

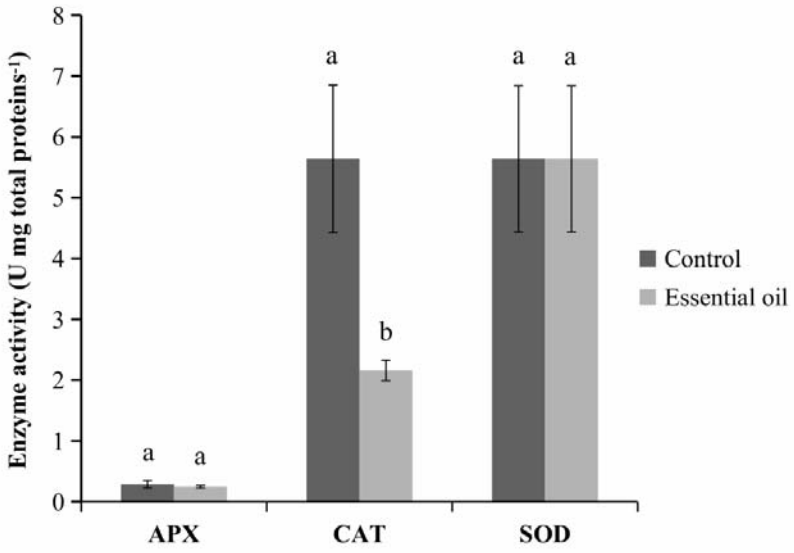

Figure 3. Activity of the enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) of *A. solani*, at 24 h after incubation with and without (control culture) essential oil from *Z. pseudodumosum*. Each bar represents the mean of independent values ± standard error. Different letters on the bars indicate that the means of the treatments differ statistically according to the Mann Whitney U analysis ($p < 0.05$).

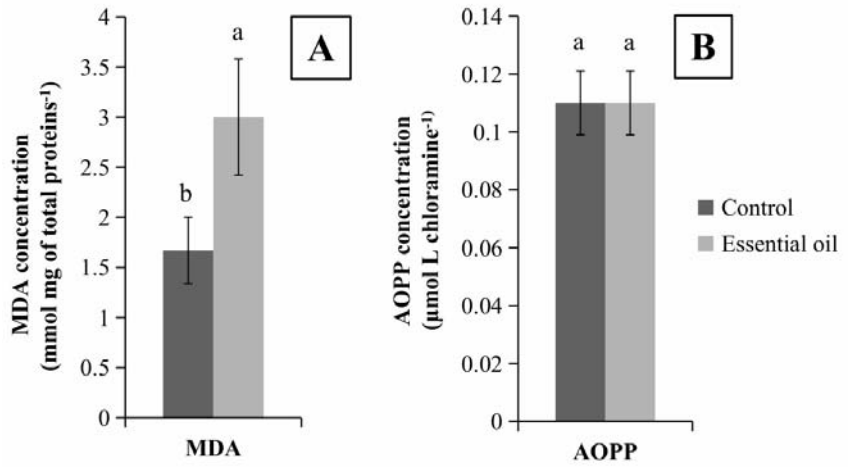

Figure 4. Concentrations of malondialdehyde (MDA; **A**) and advanced oxidation protein products (AOPP; **B**) of *A. solani*, at 24 h after incubation with and without (control culture) essential oil from *Z. pseudodumosum*. Each bar represents the mean of independent values ± standard error. Different letters on the bars indicate that the means of the treatments differ statistically according to the Mann Whitney U analysis ($p \leq 0.05$).

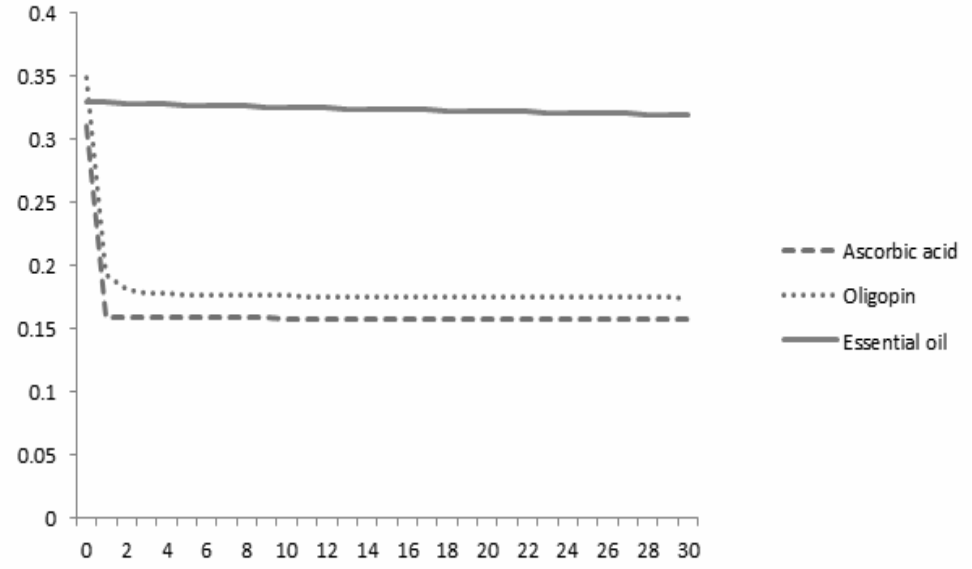

Figure 5. Time course of scavenging of the DPPH radical with different samples.

The obtained results indicate that *Z. pseudodumosum* essential oil did not exhibit radical scavenging activity *in vitro* (Figure 5). This result can be explained by the chemical nature of this essential oil, as this oil is composed almost entirely of sesquiterpene hydrocarbons which have no scavenging capacity. This also seems to confirm that the remaining unidentified portion of this oil does not seem to be constituted of phenols or other compounds, which would have a radical scavenging capacity. However, the absence of radical scavenging activity of the essential oil could be regarded as favorable when taken in relation with the results on the oxidative damage through measurements of production of malonyldialdehyde (MDA). The fact that the essential oil does not seem to have any radical scavenging capacity could mean that the mycelial growth inhibition proceeds by oxidative stress inducing damage of fungal cell membrane.

CONCLUSIONS

This study demonstrated that the essential oil from the leaves of *Z. pseudodumosum*, an endemic species growing in central Cuba, is mainly composed of sesquiterpenes hydrocarbons. A total of seventeen compounds representing 85.7% of the oil were identified by GC-MC and GC-FID and the major components were found to be β-caryophyllene, (32.01%) followed by germacrène D (14.89%) and α-selinene (8.67%). With respect to biological activity, the essential oil exhibited an interesting antifungal activity against the phytopathogenic fungus *A. solani*. This activity could be explained, at least in part, by oxidative stress induced by the essential oil, indicated by the increase in MDA production. The results of DPPH test seem to support the progress of oxidative stress which inhibits the mycelial growth. Altogether, these results confirm that the *Z. pseudodumosum* essential oil could be a natural alternative source of fungicides to treat diseases associated to the phytopathogenic fungi of the potato and tomato. However, further studies should be conducted to evaluate the efficacy of *Z. pseudodumosum* essential oil against phytopathogenic fungi under field conditions.

ACKNOWLEDGMENTS

The authors wish to thank for the scholarship offered to Lisbet Martin Cruz by Emerging Leaders in the Americas Program (ELAP), offered by Global Affairs Canada, and to the NSERC (discovery grant to T. Stevanovic) and Centre de Recherche sur les Matériaux Renouvelables (Université Laval, QC, Canada) for providing infrastructural facilities to carry out this study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Appelhans, M. S., Reichelt, N., Groppo, M., Paetzold, C. and Wen, J. 2018, Mol. Phylogenet. Evol., 126, 31-44.
2. Patiño, O. J., Prieto, J. A. and Cuca, L. E. 2011, Zanthoxylum genus as potential source of bioactive compounds, in Bioactive compounds in phytomedicine, I. Rassoli, Ed. Croatia: InTech, 185-207.
3. Beurton, C. 2008, Rutaceae. in Flora de la Republica de Cuba. W. Greuter and R. Rankin, (Eds.): A. R. Gantner Verlag KG, 134.
4. Hernandez F. A. 2014, Actividad antifúngica in vitro de extractos de hojas de especies de la familia Rutaceae frente a Mycosphaerella fijiensis Morelety Alternaria solani Sor. Universidad Central "Marta Abreu" de Las Villas, Cuba.
5. Abbas, M. F., Naz, F. and Irshad, G. 2013, Mycopath, 11(1), 45-50.
6. Duarte, Y., Pino, O., Infante, D., Sanchez, Y., Travieso, M. and Martinez, B. 2013, Rev. Protección Veg., 28(1), 54-59.
7. Farooq, M., Jabran, K., Cheema, Z. A. and Wahid, A. 2011, Pest Manag. Sci., 67(5), 493-506.
8. Adams, R. P. 2007, Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry, 4th ed. Carol Stream, IL: Allured Pub. Corp.
9. Valenzuela-Cota, D., Buitimea-Cantúa, G., Plascencia-Jatomea, M., Cinco-Moroyoqui, F., Martínez-Higuera, A. and Rosas-Burgos, E. 2019, J. Environ. Sci. Health B., 54(8), 647-654.
10. Gil-ad, N., Bar-Nun, N., Noy, T. and Mayer, A. M. 2000, FEMS Microbiol. Lett., 190(1), 121-126.
11. Choi, G., Lee, H. and Yunk, K. 1996, Pestic. Biochem. Phys., 55, 29-39.
12. Matteuci, E., Biasci, E. and Giampietro, O. 2001, Acta Diabetol., 38(4), 187-189.
13. Li, K., Zhou, R., Jia, W. W., Li, Z., Li, J., Zhang, P. and Xiao, T. 2016, J. Ethnopharmacol., 186, 351-361.
14. de Moura, N., Strapazzon, J., Loro, F., Morel, A. and Flach, A. 2005, J. Essent. Oil Res., 18(6), 627-628.
15. Pino Alea, J., Agüero, J., Marbot, R. and Fernandes, P. 2005, J. Essent. Oil Res., 17(4), 413-414.
16. Prieto, J., Patiño, O., Delgado, W., Moreno, J. and Cuca, L. 2010, Chil. J. Agr. Res., 71(1), 73-82.
17. Zhu, H., Li, Z., Jia, W., Liu, Y. and Li, K. 2018, J. Clin. Nutr. Food Sci., 1(1), 12-19.
18. Souza, J. G., Toledo, A. G., Santana, C. B., Santos, C. V., Mallmann, A. P., Silva, J. P. B. and Pinto, F. G. S. 2017, Rev. Bras. Saúde Prod. Anim., 18(3), 446-453.

19. Facundo, V., Rezende, C., Pinto, A. and de Morais, S. 2003, J. Essent. Oil Res., 15(6), 402-403.
20. Katsuhiro, T., Shigeru, M. and Toshio, Y. 1981, Agric. Biol. Chem., 45(1), 129-132.
21. Ashitani, T., Garboui, S. S., Schubert, F., Vongsombath, C., Liblikas, I., Pålsson, K. and Borg-Karlson, A. K. 2015, Exp. Appl. Acarol., 67, 595-606.
22. Bahraminejad, S., Seifolahpour, B. and Amiri, R. 2016, J. Crop. Prot., 5(4), 603-616.
23. Bayar, Y., Onaran, A., Yilar, M. and Gul, F. 2018, J. Essent. Oil Bear. Pl., 21(2), 548-555.
24. Tomazoni, E., Pansera, M., Pauletti, G., Moura, S., Ribeiro, R. and Schwambach, J. 2015, An. Acad. Bras. Ciênc., 88(2), 99-101.
25. Hamad, Y., Abobakr, Y., Salem, M., Ali, H., Al-Sarar, A. and Al-Zabib, A. 2019, BioResources, 14(2), 4489-4511.
26. Kordali, S., Usanmaz, A., Cakir, A., Komaki, A. and Ercisli, S. 2016, Chem. Biodivers.,13(1), 77-84.
27. Castro, J. C., Endo, E. H., de Souza, M. R., Zanqueta, E, B., Polonio, J. C., Pamphile, J. A., Ueda-Nakamura, T., Nakamura, C. V., Filho, B. D. and deAbreu Filho, B. A. 2017, Ind. Crop Prod., 97, 101-109.
28. Nazzaro, F., Fratianni, F., Coppola, R. and De Feo, V. 2017, Pharmaceuticals, 10(86), 1-20.
29. Babaei, G., Aliarab, A., Abroon, S., Rasmi, Y. and Aziz, S. 2018, Biomed. Pharmacother., 106, 239-246.
30. Xu, S., Zhao, X., Liu, F., Cao, Y., Wang, B., Wang, X., Yin, M., Wang, Q. and Feng, X. 2018, Pest Manag. Sci., 74(12), 2716-2723.
31. Jia, M., Jiang, X., Xu, J., Di, W., Shi, Y. and Liu, Y. 2018, Acta Physiol. Plant., 40, 37.
32. Huang, L., Luo, D., Fu, R., Yan, B., Wang, X., Shu, J., Deng, J. and Huang, H. 2019, Fitoterapia, 137, 104247.
33. Ramachandhiran, D., Sankaranarayanan, C., Murali R., Babukumar, S. and Vinothkumar, V. 2019, Arch. Physiol. Biochem., 4, 1-15.