



# Essential oil composition and antibacterial activity of *Pteridium aquilinum* (L.) Kuhn

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## Original Article

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**Introduction:** The present work aims to study the chemical composition of *Pteridium aquilinum* (L.) Kuhn essential oil and its antibacterial activity against three important phytopathogenic Gram-negative bacteria: *Erwinia amylovora*, *Pectobacterium carotovorum* subsp. *carotovorum*, and *Pseudomonas savastanoi* pv. *savastanoi*. **Methods:** The chemical composition of *P. aquilinum* L. essential oil produced by hydrodistillation was determined by gas chromatography–mass spectrometry. The antibacterial activity was tested using disk diffusion method and by determination of minimum inhibitory concentration values. The major components were linalool (10.29%), carvacrol (8.15%), benzaldehyde (5.95%), 2-undecanone (5.32%), and cuminaldehyde (4.57%). **Results:** The essential oil tested revealed a powerful antibacterial effect against all tested strains, with inhibition zone diameters ranging from  $32.0 \pm 0.58$  to  $33.7 \pm 0.88$  mm. **Discussion:** *P. aquilinum* EO contained 32.86% of oxygenated monoterpenes, which are known for their very powerful antimicrobial activities. The minimum inhibitory concentration values showed that *P. aquilinum* essential oil has very strong activity against *E. amylovora* (0.625  $\mu$ l/ml), followed by *P. carotovorum* subsp. *carotovorum* (2.50  $\mu$ l/ml) and *P. savastanoi* pv. *savastanoi* (5.00  $\mu$ l/ml). The results obtained could contribute to the development of new potential agents for the control of bacterial diseases.

## INTRODUCTION

A variety of pathogenic bacteria present in the environment cause major problems in the agricultural domain and contribute to substantial losses of crop yields (Gomez et al., 2015). Most of these phytopathogenic microorganisms are Gram-negative bacteria and resistant to usual treatment (Zarai et al., 2011). Bacterial diseases caused by *Erwinia amylovora* (fire blight on *Rosaceae*), *Pectobacterium carotovorum* subsp. *carotovorum* (soft rot of potatoes), and *Pseudomonas savastanoi* pv. *savastanoi* (olive knot in Mediterranean countries) are classified among the most dangerous phytopathogenic bacteria (Bhardwaj & Laura, 2008; Karami-Osboo et al., 2010; Quesada et al., 2012). Actually, these diseases are controlled by pesticides and synthetic antibiotics. However, many of these treatments cause undesirable effects on the environment and on the health of human and mammals (Gomez et al., 2015; Kotan et al., 2014). Therefore, it is important to evaluate and develop new natural bioactive compounds as alternative biological control agents such as essential oils (EOs). Their chemical composition is eminently variable. Despite this heterogeneity, these metabolites have marked properties that are valued in many areas, including aromatherapy and cosmetics to which biocontrol of bio-aggressors is added (Regnault-Roger et al., 2008). In recent years, numerous studies have reported on the efficacy of EOs for crop protection as antibacterial agent (Bajpai et al., 2010; Gomez et al., 2015; Kotan et al., 2014; Pandey et al., 2012).

Ferns consist of a group of about 12,000 different species and more than 250 genera (Fernández et al., 2011). *Pteridium* is an important genus belonging to the *Dennstaedtiaceae* (Bernh.) family (Smith et al., 2006). *Pteridium aquilinum* (L.) Kuhn, also known as the bracken fern, has a cosmopolitan distribution (Cody & Crompton, 1975).

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This plant can grow to a height of 2 m. It has a thick creeping rhizome and delta-shaped fronds with 0.5–1.5 m long, divided into pinnae, which are subdivided into pinnules. The stipe is long and can reach 55 cm and a long brown stipe up to 55 cm (Adou & Ipou Ipou, 2007; Yan et al., 2013). Several studies demonstrated that *P. aquilinum* L. metabolites show antibacterial (Hassan et al., 2007; Kardong et al., 2013), antifungal (Sahayaraj et al., 2009), insecticidal (Selvaraj et al., 2005), antioxidant (Kardong et al., 2013; Panneerselvam et al., 2015), and antimalarial (Panneerselvam et al., 2015) activities. The chemical composition of *P. aquilinum* L. EO and its biological activities are little known (Halarewicz & Szumny, 2010; Froissarda et al., 2011; Nwiloh et al., 2014). The aim of the present work is to determine the bioactive compounds of the EO of *P. aquilinum* L. and evaluate its antibacterial properties against three plant pathogenic bacteria: *E. amylovora*, *P. carotovorum* subsp. *carotovorum*, and *P. savastanoi* pv. *savastanoi*.

## MATERIALS AND METHODS

### Plant material

*P. aquilinum* L. fronds were collected near Ben-Ali station, Chrea, at an altitude of 700 m, 13.4 km from Blida city (Algeria). The fresh fronds were washed in water, dried in the shade, and ground to powder in a grinder. The plant was identified in the Department of Botany, National Higher School of Agronomy El-Harrach, Algeria in which voucher specimens were deposited at the Herbarium.

### EO extraction

About 100 g of dried fronds of *P. aquilinum* L. were subjected to hydrodistillation in a Clevenger-type apparatus for 4 hr. The EO was separated from the water by decantation, dried over anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), and stored for analysis at a temperature of 4 °C.

### Gas chromatography–mass spectrometry analysis

The chemical composition of EO was analyzed by gas chromatography–mass spectrometry (GC/MS) using an Agilent gas chromatograph (HP 6850) coupled with the mass selective detector of an Agilent HP 5973 mass spectrometer operating in electron ionization mode. A DB-5 fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm) was used, and the chromatography conditions were as follows: injector and transfer line temperatures: 250 and 280 °C, respectively; oven temperature: 60 °C, increasing by 3 °C/min to 245 °C, and then held stable for 4 min; vector gas: helium at a flow rate of 1.3 ml/min; injection volume: 2 µl (0.5% in hexane solution) in splitless mode. The conditions of the mass unit were: ion source temperature 230 °C; mass spectrum recorded at an ionization voltage of 70 eV with a mass scan range of 35–250 m/z.

The components of the EO were identified by comparing their mass spectra fragmentation with those from Wiley 257 and Adams mass spectra libraries and by comparing their corresponding retention indices (RIs) with other given in the

literature (Adams, 2007). The RIs of the EO components were calculated experimentally using the retention time of the homologous *n*-alkane series (C8–C21), injected in the same chromatographic conditions. The percentage of EO components was computed from the GC/MS peak areas without any correction factors.

### Antibacterial activity

**Bacterial strains.** Three phytopathogenic Gram-negative bacteria: *E. amylovora*, *P. carotovorum* subsp. *carotovorum*, and *P. savastanoi* pv. *savastanoi* were tested. The cultures of these bacteria were obtained from the National Institute for Plant Protection, El-Harrach, Algeria.

**Disk diffusion method.** The antibacterial activity of *P. aquilinum* L. EO was evaluated by the disk diffusion method (Kotan et al., 2010; Murray et al., 1995). Yeast peptone glucose agar medium was used for *E. amylovora* and *P. carotovorum* subsp. *carotovorum*, and King B medium for *P. savastanoi* pv. *savastanoi*. Disks (6 mm in diameter) were impregnated with 12.5 µl of the EO and deposited in the middle of the dishes previously surface-streaked with microbial suspension (10<sup>8</sup> UFC/ml). Streptomycin (10 µg/disk) was used as positive control. All the dishes were incubated at 27 ± 2 °C for 48 hr. All the tests of this experiment were performed in triplicate.

**Bactericidal and bacteriostatic effects.** Bactericidal and bacteriostatic effects were determined according to the technique described by Kotan et al. (2010). Nutrient agar samples from areas inhibited around the disks were placed in a nutrient broth without EO and incubated at 27 ± 2 °C for 48 hr. After 48 hr, if no bacterial growth was observed, the effect was considered as bactericidal. If bacterial growth was observed in the broth culture, the effect was considered as bacteriostatic.

**Minimum inhibitory concentration (MIC).** The MICs were determined by the agar diffusion method reported in the studies of Kotan et al. (2010, 2014). Solutions were prepared by serial dilution of *P. aquilinum* EO in 10% of dimethyl sulfate (DMSO) to obtain concentrations decreasing from 40 to 0.315 µl/ml. Disks impregnated with 12.5 µl of the solutions under test were placed on the agar previously inoculated with bacterial cultures and were incubated at 27 ± 2 °C for 48 hr. The weakest concentration showing a clear inhibition zone was taken as the MIC. DMSO was used as negative control. All the tests were performed in triplicate.

### Statistical analyses

The effects of the EO on bacterial growth results were evaluated by one-way ANOVA and Tukey's test with *p* < .05 as a significance level. The data were analyzed using the Systat 7.0 package for Windows (Chicago, IL, USA).

## RESULTS

### Chemical composition of EO

The EO (dark yellow) was obtained by hydrodistillation of *P. aquilinum* fronds, with a yield of 0.025% (w/w, based on dry weight). The composition of this oil was analyzed

Table 1. Chemical composition (%) of *P. aquilinum* L. essential oil

Components	RI <sub>E</sub>	RI <sub>L</sub>	%
2-Hexenal	834	846	0.12
Heptanal	899	901	0.11
Benzaldehyde	959	952	5.95
1-Octen-3-ol	975	974	0.26
2-Pentylfuran	985	984	0.14
3-Octanol	995	988	0.19
<i>n</i> -Octanal	1,001	998	0.09
Limonene	1,026	1,024	0.07
β-Ocimene	1,041	1,044	0.09
2-Octenal	1,054	1,049	0.16
<i>cis</i> -Linalool oxide (furanoid)	1,066	1,067	0.05
Terpinolene	1,082	1,086	0.12
Linalool	1,098	1,095	10.29
Nonanal	1,102	1,100	2.67
<i>neo</i> -3-Thujanol	1,151	1,149	0.14
Nonanal	1,156	1,157	0.33
4-Terpineol	1,178	1,174	0.11
Naphthalene	1,182	1,178	1.61
α-Terpineol	1,194	1,186	3.51
<i>n</i> -Decanal	1,203	1,201	0.18
Nerol	1,222	1,227	0.98
Pulegone	1,234	1,233	0.47
Cuminic aldehyde	1,240	1,238	4.57
Geraniol	1,248	1,249	3.30
Isopulegyl acetate	1,272	1,275	0.12
Vitispirane	1,274	1,272	0.22
<i>trans</i> -Anethole	1,284	1,282	1.37
2-Undecanone	1,289	1,293	5.32
Thymol	1,296	1,289	0.30
2-Undecanol	1,301	1,301	0.15
Carvacrol	1,304	1,298	8.15
Theaspirane B	1,309	1,299	1.52
<i>trans,trans</i> -2,4-Decadienal	1,316	1,315	0.58
<i>trans</i> -Piperitol acetate	1,341	1,343	0.18
<i>trans</i> -β-Damascenone	1,372	1,383	0.58
β-Caryophyllene	1,412	1,417	1.07
α-Ionone	1,415	1,428	0.17
Geranyl acetone	1,442	1,453	0.69
α-Humulene	1,448	1,452	0.28
β-Ionone	1,472	1,487	3.20
Eremophilene	1,492	1,498	0.29
β-Bisabolene	1,501	1,505	0.27
Tridecanal	1,507	1,509	1.50
Calamenene	1,515	1,528	0.70
α-Calacorene	1,534	1,544	0.20
Spathulenol	1,571	1,577	0.35
Caryophyllene oxide	1,573	1,582	0.88
Veridiflorol	1,587	1,592	0.11
Longiborneol	1,601	1,599	0.14
Dill apiol	1,612	1,620	0.25
β-Tumerone	1,658	1,668	0.40
2-Pentadecanone	1,693	1,697	0.19
<i>epi</i> -Cyclocolorone	1,774	1,774	2.07
Farnesyl acetone	1,901	1,913	0.54
Isophytol	1,939	1,946	0.32
Phytol	2,101	2,105	0.21
Monoterpene hydrocarbons			0.28
Oxygen-containing monoterpenes			32.86
Sesquiterpene hydrocarbons			4.42

Table 1. (Continued)

Components	RI <sub>E</sub>	RI <sub>L</sub>	%
Oxygen-containing sesquiterpenes			4.49
Others			27.39
Total identified			69.44

Note. RI<sub>E</sub>: experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes (C<sub>8</sub>–C<sub>21</sub>); RI<sub>L</sub>: retention indices literature data (Adams, 2007; Boulanger & Crouzet, 2001; Jantan et al., 2003; Pino et al., 2004, 2005; Tzakou et al., 2004).

<sup>a</sup>Compounds listed in order of elution on DB-5 column.

by GC-MS that has been summarized in Table 1, where compounds are listed according to their elution on a DB-5 column in parallel with their RI and their relative percentage. A total of 56 components were identified, representing 69.44% of the total detected constituents with predominance of oxygenated monoterpenes followed by non-terpene compounds. The results showed that linalool and carvacrol were found to be the major compounds in the EO of Algerian *P. aquilinum* followed by benzaldehyde, 2-undecanone, and cumin aldehyde. In addition, other components such as α-terpineol, geraniol, β-ionone, and nonanal were detected with notable amounts in this oil.

#### Antibacterial activity of EO

To our knowledge, this is the first report on the antibacterial activity of *P. aquilinum* EO against phytopathogenic bacteria: *E. amylovora*, *P. carotovorum* subsp. *Carotovorum*, and *P. savastanoi* pv. *savastanoi*. The inhibition zone diameter and MIC values of the EO against different phytopathogenic bacteria are presented in Table 2. On the basis of Rota et al.'s (2008) classification of the levels of EOs antibacterial activity, *P. aquilinum* oil at 12.5 μl/disk revealed a remarkable antibacterial–bactericidal effect against all tested strains (32.0–33.7 mm). Streptomycin showed strong antibacterial activity against *P. carotovorum* subsp. *carotovorum* and moderate activity against *E. amylovora* and *P. savastanoi* pv. *savastanoi*. EO of *P. aquilinum* had a bactericidal activity against all three strains tested, and no difference was recorded in its effects on the three strains ( $p > .05$ ). The antibacterial activity of the EO was highly significant with a large inhibition zone compared to that of streptomycin ( $p < .05$ ). The MIC values given in Table 2 show that *P. aquilinum* EO presents very strong activity against *E. amylovora*, with a value of 0.62 μl/ml, followed by *P. carotovorum* subsp. *carotovorum* with 2.50 μl/ml and finally *P. savastanoi* pv. *savastanoi* with 5.00 μl/ml.

#### DISCUSSION

The chromatographic profile obtained in this study was distinct to that of the previous reports (Halarewicz & Szumny, 2010; Nwiloh et al., 2014). In the EO obtained from *P. aquilinum* fronds originated from Poland, benzaldehyde (44%–50%) was dominated. The chromatographic analysis of fiddleheads oil of the same species from Nigeria

Table 2. Antibacterial activity (DI/MIC) of the EO of *P. aquilinum* L.

Strains	EO		Streptomycin	DMSO
	DI [(mm) ± SE] <sup>a</sup>	MIC	DI [(mm) ± SE] <sup>b</sup>	DI [(mm) ± SE] <sup>c</sup>
<i>E. amylovora</i>	32.0 ± 0.58*	0.625	15.0 ± 0.58	6.00 ± 0.00
<i>P. carotovorum</i> subsp. <i>carotovorum</i>	33.7* ± 0.88*	2.50	21.3 ± 0.33	6.00 ± 0.00
<i>P. savastanoi</i> pv. <i>savastanoi</i>	33.0 ± 0.58*	5.00	15.7 ± 0.33	6.00 ± 0.00

Note. MIC: minimum inhibitory concentration (µl/ml); EO: essential oil; DMSO: dimethyl sulfate.

<sup>a</sup>DI: diameters of inhibition zone (mm) of EO (12.5 µl/disk) including the disk diameter (6 mm; mean of triplicates). <sup>b</sup>DI: diameters of inhibition zone (mm) of Streptomycin (10 µg/disk) including the disk diameter (6 mm). <sup>c</sup>DI: diameters of inhibition zone (mm) of DMSO including the disk diameter (6 mm).

\*Bactericidal effect observed.

was revealed the presence of alkane (86.60%), monoterpene (3.20%), and sesquiterpenes (2.40%). The EO consisted mainly of tetratriacontane (12.40%). According to Sangwan et al. (2001), the yield and the composition of the EOs of the same species can be influenced by ontogenesis, environmental factors, and the harvesting periods and sites. Consequently, a significant alteration of the biochemical pathways and the physiological processes appears to modify the biosynthesis of the EO.

A review of the literature showed that a relationship exists between antimicrobial activity and the chemical composition of the EO, the chemical structures of the functional groups and their configurations, the proportions in which they are present, and the interactions among them (Dorman & Deans, 2000). The high antibacterial potential of EO of *P. aquilinum* could be explained by the presence of certain major and/or minor components. As shown in Table 1, *P. aquilinum* EO contained 32.86% of oxygenated monoterpenes, which are known for their very powerful antimicrobial activities, especially the alcoholic- and phenolic-oxygenated terpenes (Kotan et al., 2007a; Zengin & Baysal, 2014). It is important to note that the percentages of linalool and carvacrol were 10.29% and 8.15%, respectively. These two substances have also been tested for the antibacterial activity. Linalool has a broad antibacterial spectrum against several pathogenic and phytopathogenic bacteria (Bassolé et al., 2010; Dadasoglu et al., 2011; Kotan et al., 2007a; Park et al., 2012; Pattnaik et al., 1997). It has also been demonstrated that carvacrol is a powerful component acting against all the phytopathogenic strains tested by Kotan et al. (2007b, 2010, 2014), Karami-Osboo et al. (2010), and Dadasoglu et al. (2011). Certain studies suggest that carvacrol is capable of disintegrating the outer membrane of Gram-negative bacteria and increasing the permeability of the cytoplasmic membrane to adenosine triphosphate (Helander et al., 1998). Carvacrol also acts as a cation (H<sup>+</sup> and K<sup>+</sup>) exchanger, dissipating the ion gradient and thus leading to a deterioration of the essential processes of the cell and finally to its death (Ultee et al., 1999). Linalool and α-terpineol alter the permeability of the outer membrane and the function of the cell membrane, leading to leakage of intracellular materials (Zengin & Baysal, 2014). The activity found for our EO may also be due to the presence of benzaldehyde (5.95%). This compound is an antibacterial substance that interacts with the surface of the cell and leads to cell death by disintegration of the cell membrane and release of the intracellular components

(Alamri et al., 2012). Thus, the activity observed for the EO may be linked with the presence of other substances already described in the literature as possessing antibacterial properties: 2-undecanone (Reddy & Al-Rajab, 2016), cuminaldehyde (Iacobellis et al., 2005), and α-terpineol (Zengin & Baysal, 2014). It should not be forgotten that the minor components of EOs, and their synergistic or antagonistic interactions, can also have an influence on the antibacterial effects (Kotan et al., 2010).

## CONCLUSION FOR FUTURE BIOLOGY

This study showed that the chemical profile of *P. aquilinum* EO was characterized by linalool, carvacrol, benzaldehyde, and 2-undecanone as the main compounds. The investigated oil possesses a powerful antibacterial and bactericidal activity against the three Gram-negative phytopathogenic bacteria tested. Toxicity by chemical pesticides is of varying intensity. It may induce researchers to look for new control alternatives such as botanical biopesticides due their wide and efficient spectrum and low toxicity. Very little work has been carried out on the EO of *P. aquilinum* as a biopesticide. These results may be of significant interest. *P. aquilinum* EO may be suggested as a potential new potential source of a natural antimicrobial and could be constituted as an alternative to chemical products.

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