

# Phenolic Composition, Antioxidant Capacity, and Antibacterial Activity of Hydroalcoholic Extracts from *Cupressus sempervirens* L. and *Cupressus arizonica* L. Against *Pseudomonas savastanoi* pv. *Savastanoi*

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Received : 18-09-2025

Accepted : 02-12-2025

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## ABSTRACT

The growing demand for environmentally friendly alternatives to synthetic pesticides has intensified interest in plant-derived bioactive compounds for sustainable disease management. In this context, the present study investigated the antioxidant and antibacterial properties of hydroalcoholic leaf extracts obtained from *Cupressus sempervirens* L. and *Cupressus arizonica* L. against the phytopathogenic bacterium *Pseudomonas savastanoi* pv. *savastanoi* ; the causal agent of olive knot disease.

Hydroalcoholic extraction (70% methanol) yielded 15.34% and 11.31% for *C. sempervirens* and *C. arizonica*, respectively. Quantification of total phenolic compounds using the folin-ciocalteu method revealed high polyphenol contents in both species, reaching 2.128 and 2.114 mg gallic acid equivalents (GAE)/g extract for *C. sempervirens* and *C. arizonica*, respectively, with no significant difference between them.

The antioxidant activity was evaluated using the DPPH radical scavenging assay. Both extracts exhibited strong antiradical activity, which increased as concentration decreased. At 0.115 g/ml, DPPH reduction percentages reached 78.36% for *C. sempervirens* and 91.09% for *C. arizonica*. Probit analysis estimated IC values of 0.297 and 0.512 g/ml for *C. sempervirens* and *C. arizonica*, respectively.

Antibacterial activity was assessed using the agar disc diffusion method. Both extracts effectively inhibited the growth of *P. savastanoi* pv. *Savastanoi*, with inhibition zones increasing proportionally to extract concentration. Probit regression analysis estimated LD50 values of 3.44 g/mL and 1.88 g/mL and LD99 values of 62.72 g/mL and 14.15 g/mL for *C. Sempervirens* and *C. arizonica*, respectively.

Overall, the results demonstrate that both cypress species represent promising natural sources of bioactive compounds with antioxidant and antibacterial properties. Considering extraction yield and lethal dose requirements, *C. arizonica* appears to be the more economically advantageous species for the development of plant-based bioproducts targeting olive knot disease management.

**Keywords :** *Cupressus arizonica* ; *cupressus sempervirens*; *pseudomonas savastanoi* ; polyphenols ; antioxidant activity ; antibacterial activity ; biocontrol ; bioproducts.

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## 1. INTRODUCTION

The olive tree (*Olea europaea* L.) is deeply woven into the cultural and economic fabric of the Mediterranean region. Yet its productivity is constantly challenged by a range of phytopathological threats, and few are as destructive as olive knot disease. Caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi*, this disease manifests as hyperplastic knots on branches, twigs, and trunks, growths that compromise vascular transport, drain the tree's resources, and ultimately reduce yield.

For decades, management has relied on copper-based compounds and preventive cultural practices. But these approaches are increasingly coming under scrutiny. Copper accumulation in soils, the emergence of resistant

bacterial strains, and tightening regulatory restrictions on synthetic pesticides have all driven a search for alternatives that are both effective and environmentally benign.

Plant extracts have emerged as a particularly attractive avenue. Rich in secondary metabolites, especially phenolic compounds, they offer multiple mechanisms of antimicrobial action: membrane disruption, enzyme inhibition, and interference with core metabolic processes. Their antioxidant properties add another layer of biological relevance, particularly in plant-pathogen interactions where oxidative stress plays a central role.

Species within the genus *Cupressus* are known to accumulate a wide array of these bioactive molecules, including flavonoids, tannins, phenolic acids, and terpenoids. Numerous studies have documented antimicrobial, anti-inflammatory, and antioxidant activities in cypress extracts, but surprisingly little work has focused on their potential against phytopathogenic bacteria, and even less on the specific pathogen responsible for olive knot disease.

This study was designed to fill that gap. We compared the phenolic composition, antioxidant capacity, and antibacterial activity of hydroalcoholic leaf extracts from *Cupressus sempervirens* and *Cupressus arizonica* against *P. savastanoi* pv. *savastanoi*, with the broader aim of identifying candidate species for developing greener biocontrol products.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Leaves of *C. sempervirens* L. and *C. arizonica* L. were collected in April 2021 at the Department of Biotechnology, University of Blida 1, Algeria. Botanical identification was carried out by Dr. Hoceme Degaïchia (Research Center for Agropastoralism, Djelfa, Algeria). The plant material was air-dried in the shade, ground to a fine powder, and stored until extraction.

### 2.2 Bacterial Strain

The target microorganism, *Pseudomonas savastanoi* pv. *savastanoi*, was isolated from olive tissues at an early stage of infection. Isolation and identification were performed by Dr. Dounia Sadak at the National Institute of Plant Protection (INPV), Boufarik Station, Algeria.

### 2.3 Preparation of Hydroalcoholic Extract (Degaïchia et al., 2022; Romaniand al., 2006)

Ten grams of dried plant powder were macerated in 100 mL of 70% methanol (v/v) under continuous agitation for 24 hours at room temperature in the dark. After filtration, the solvent was removed under reduced pressure using a rotary evaporator set at 45°C. The resulting dry extracts were stored at 4°C until analysis. Extraction yield was calculated as mentioned in Falleh and al., (2008):  $\text{Yield (\%)} = (\text{Mass of dry extract} / \text{Mass of dry plant material}) \times 100$

### 2.4 Determination of Total Phenolic Content (Singleton and Rossi (1965))

Total phenolic content was quantified spectrophotometrically using the Folin-Ciocalteu reagent. Absorbance was recorded at 765 nm, and results were expressed as gallic acid equivalents (mg GAE g<sup>-1</sup> extract).

### 2.5 DPPH Radical Scavenging Assay

Antioxidant activity was evaluated through the DPPH method. Extract concentrations ranging from 0.115 to 0.575 g mL<sup>-1</sup> were tested. After 30 minutes of incubation in the dark, absorbance was measured at 515 nm. The percentage of DPPH inhibition was calculated as:

$$\text{DPPH Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

### 2.6 Antibacterial Activity (Shunying and al., 2005 ; Celiktas and al., 2007 ; Bssaibis and al., 2009 ; Ngameni and al., 2009)

Antibacterial testing followed the agar disc diffusion method. King B medium was inoculated with bacterial suspensions adjusted to 10<sup>8</sup> CFU mL<sup>-1</sup>. Sterile paper discs (6 mm diameter) were impregnated with 10 µL of each extract concentration and placed on the inoculated plates. Incubation was carried out at 38°C for 24 to 48 hours, after which inhibition zone diameters and surface areas were measured.

## 2.7 Statistical Analysis

All experiments were conducted in triplicate. Data were processed using SPSS 21.0. Normality was confirmed with the Shapiro-Wilk test, and comparisons were made using Student's t-test and one-way ANOVA followed by Tukey's post hoc test ( $p < 0.05$ ).  $IC_{50}$ ,  $LD_{50}$ , and  $LD_{99}$  values were estimated through Probit regression analysis.

## 3. RESULTS

### 3.1 Extraction Yield

Hydroalcoholic extraction produced yields of 15.34% for *C. sempervirens* and 11.31% for *C. arizonica*. The higher recovery from *C. sempervirens* suggests a greater pool of extractable biomass under these conditions, likely reflecting species-level differences in tissue composition and metabolite accumulation.

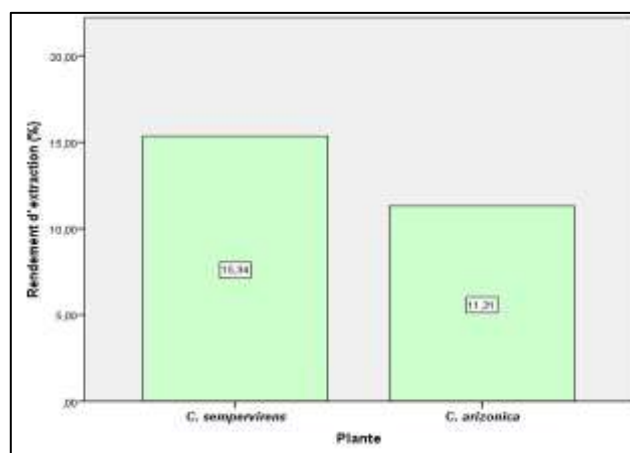


Figure 1: Extraction yield (%) of hydroalcoholic phenolic extracts from *Cupressus sempervirens* and *Cupressus arizonica*

### 3.2. Total Phenolic Content

Both extracts contained substantial amounts of phenolic compounds: 2.128 mg GAE  $g^{-1}$  for *C. sempervirens* and 2.114 mg GAE  $g^{-1}$  for *C. arizonica*. The difference between the two species was not statistically significant, indicating a broadly comparable phenolic load.

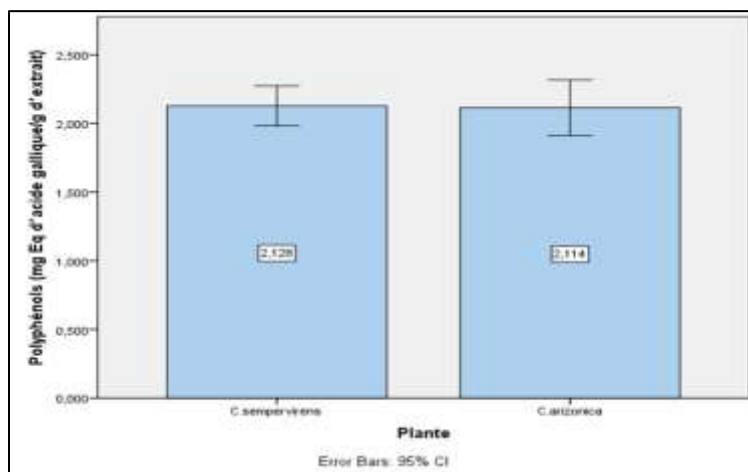


Figure 2: Total phenolic content, expressed as gallic acid equivalents (GAE), in aqueous methanolic leaf extracts of *Cupressus sempervirens* L. and *Cupressus arizonica* L.

### 3.3. Antioxidant Activity

Both extracts displayed pronounced DPPH scavenging capacity. Interestingly, the antioxidant effect was strongest at the lowest concentration tested and diminished as concentration increased.

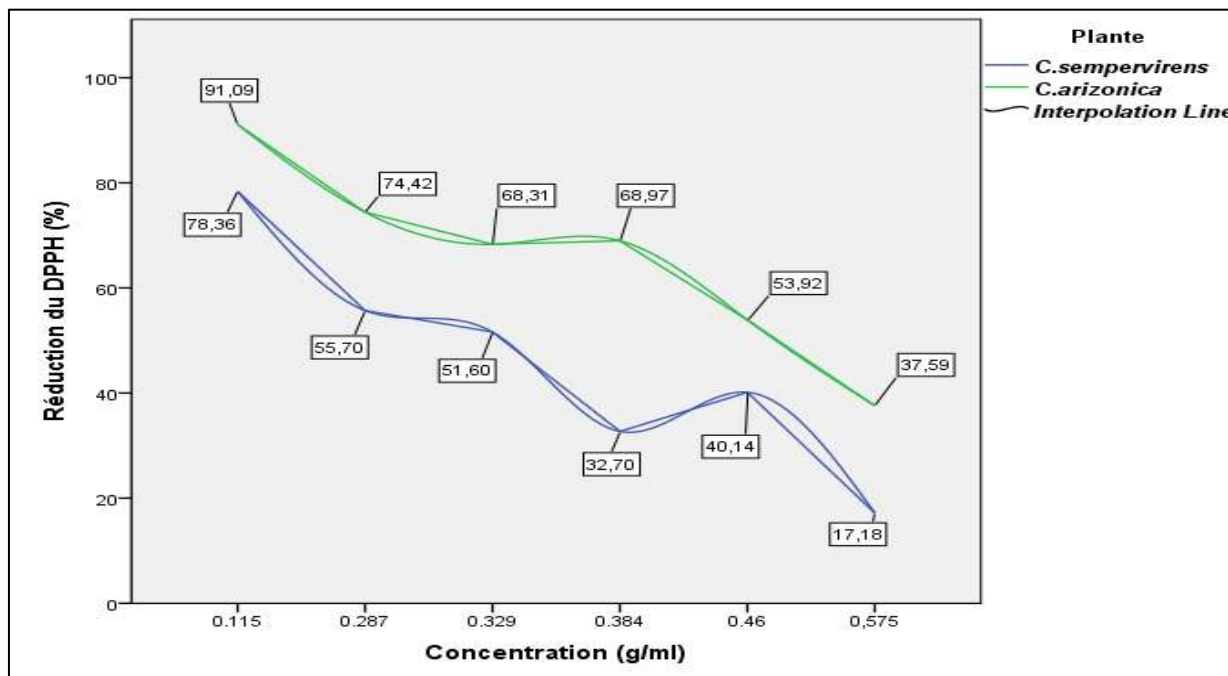


Figure 3: DPPH radical scavenging activity of hydroalcoholic extracts from *Cupressus sempervirens* and *Cupressus arizonica*.

At 0.115 g mL<sup>-1</sup>, inhibition reached 78.36% (*C. sempervirens*) and 91.09% (*C. arizonica*). At the highest concentration (0.575 g mL<sup>-1</sup>), these values dropped to 17.18% and 37.59%, respectively. This inverse pattern, while counterintuitive at first glance, has occasionally been noted in complex plant extracts and may reflect concentration-dependent molecular interactions or differential activation of antioxidant mechanisms. Probit analysis placed the IC<sub>50</sub> at 0.297 g mL<sup>-1</sup> for *C. sempervirens* and 0.512 g mL<sup>-1</sup> for *C. arizonica*.

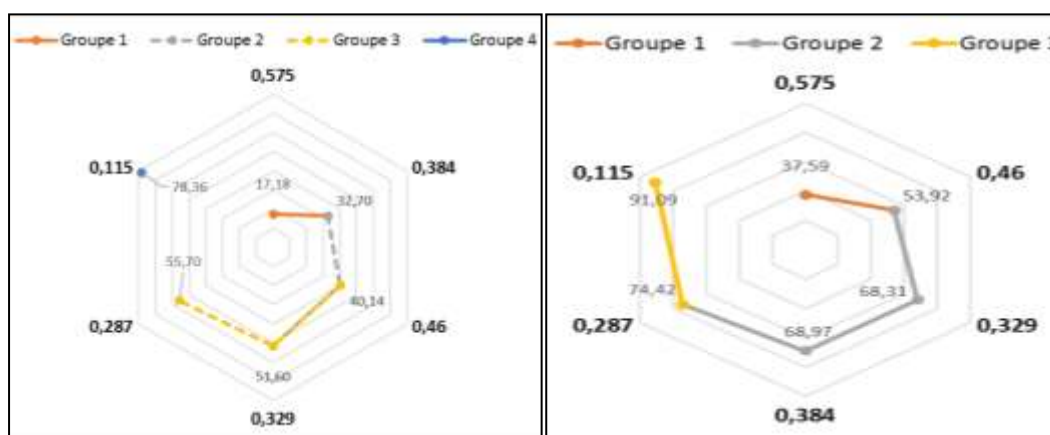
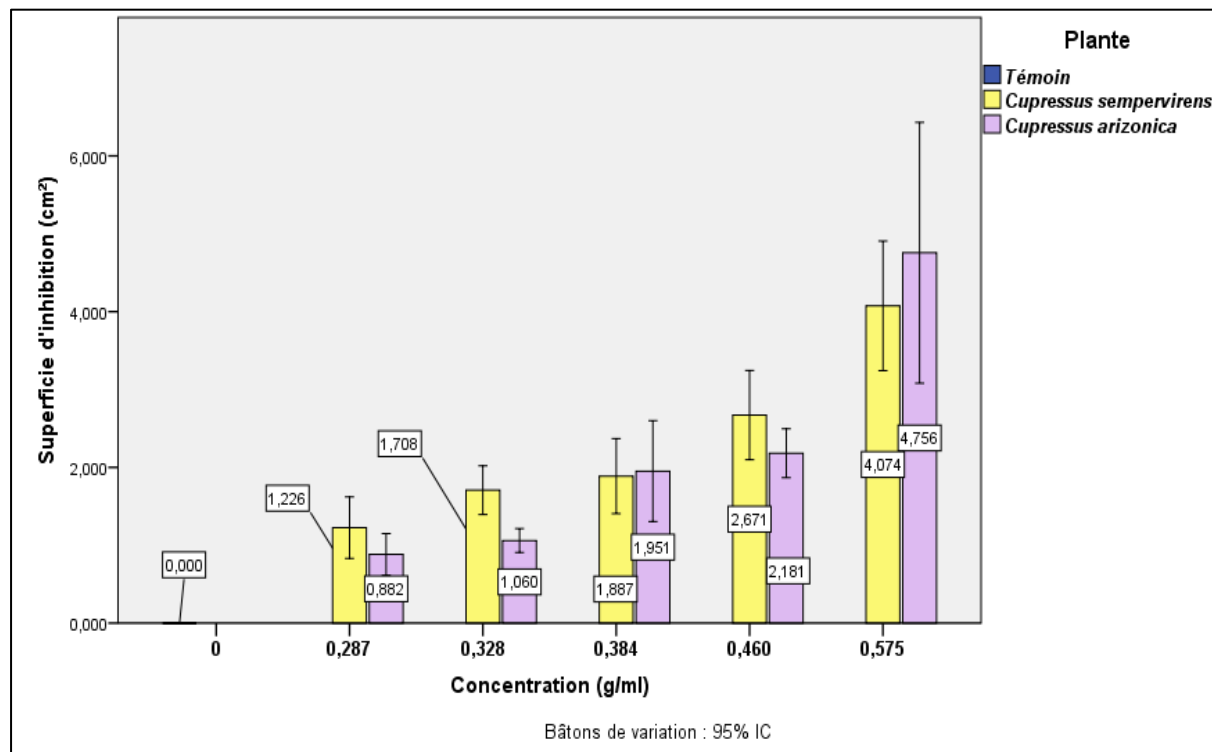


Figure 4: Homogeneous grouping of *C. sempervirens* and *C. arizonica* extract doses based on their DPPH radical scavenging activity (%).

### 3.4. Antibacterial Activity

Both extracts inhibited the growth of *P. savastanoi* pv. *savastanoi*, and the effect was clearly concentration-dependent. At 0.287 g mL<sup>-1</sup>, inhibition surface areas measured 1.226 cm<sup>2</sup> for *C. sempervirens* and 0.882 cm<sup>2</sup> for *C. arizonica*. At the maximum tested concentration (0.575 g mL<sup>-1</sup>), these values rose to 4.074 cm<sup>2</sup> and 4.756 cm<sup>2</sup>, respectively. Statistical analysis confirmed that the differences across concentrations were significant.



**Figure 5:** Variation in the inhibition area (cm<sup>2</sup>) of *Pseudomonas savastanoi* pv. *savastanoi* as a function of hydroalcoholic extract concentration from *Cupressus sempervirens* and *Cupressus arizonica*. Error bars indicate 95% confidence intervals.

The antibacterial activity of the hydroalcoholic extracts of *Cupressus sempervirens* and *Cupressus arizonica* against *Pseudomonas savastanoi* pv. *savastanoi* exhibited a clear concentration-dependent response. As extract concentration increased from 0.287 to 0.575 g mL<sup>-1</sup>, bacterial growth inhibition became progressively more pronounced, resulting in a substantial enlargement of the inhibition zones. At the lowest tested concentration (0.287 g mL<sup>-1</sup>), inhibition areas of 1.226 and 0.882 cm<sup>2</sup> were recorded for *C. sempervirens* and *C. arizonica*, respectively. Increasing the concentration to 0.575 g mL<sup>-1</sup> significantly enhanced antibacterial activity, yielding inhibition areas of 4.074 cm<sup>2</sup> for *C. sempervirens* and 4.756 cm<sup>2</sup> for *C. arizonica*. Overall, both extracts displayed comparable antibacterial efficacy across most concentrations tested. However, at 0.328 g mL<sup>-1</sup>, the extract of *C. sempervirens* produced significantly greater inhibition than that of *C. arizonica*, as confirmed by Student's *t*-test. Further statistical evaluation using one-way ANOVA followed by Tukey's post-hoc test revealed significant differences among concentration levels and allowed the classification of inhibition areas into homogeneous groups. For *C. arizonica*, two homogeneous groups were identified, with concentrations of 0.287, 0.328, 0.384, and 0.460 g mL<sup>-1</sup> forming the first group, while the highest concentration (0.575 g mL<sup>-1</sup>) constituted a distinct second group. In contrast, the inhibition response of *C. sempervirens* was distributed into three homogeneous groups: the first included concentrations of 0.287, 0.328, and 0.384 g mL<sup>-1</sup>; the second comprised 0.328, 0.384, and 0.460 g mL<sup>-1</sup>; whereas the highest concentration (0.575 g mL<sup>-1</sup>) formed an independent third group. These findings demonstrate that increasing extract concentration significantly enhances antibacterial efficacy and highlight the strong inhibitory potential of both cypress species against *P. savastanoi* pv. *savastanoi*.

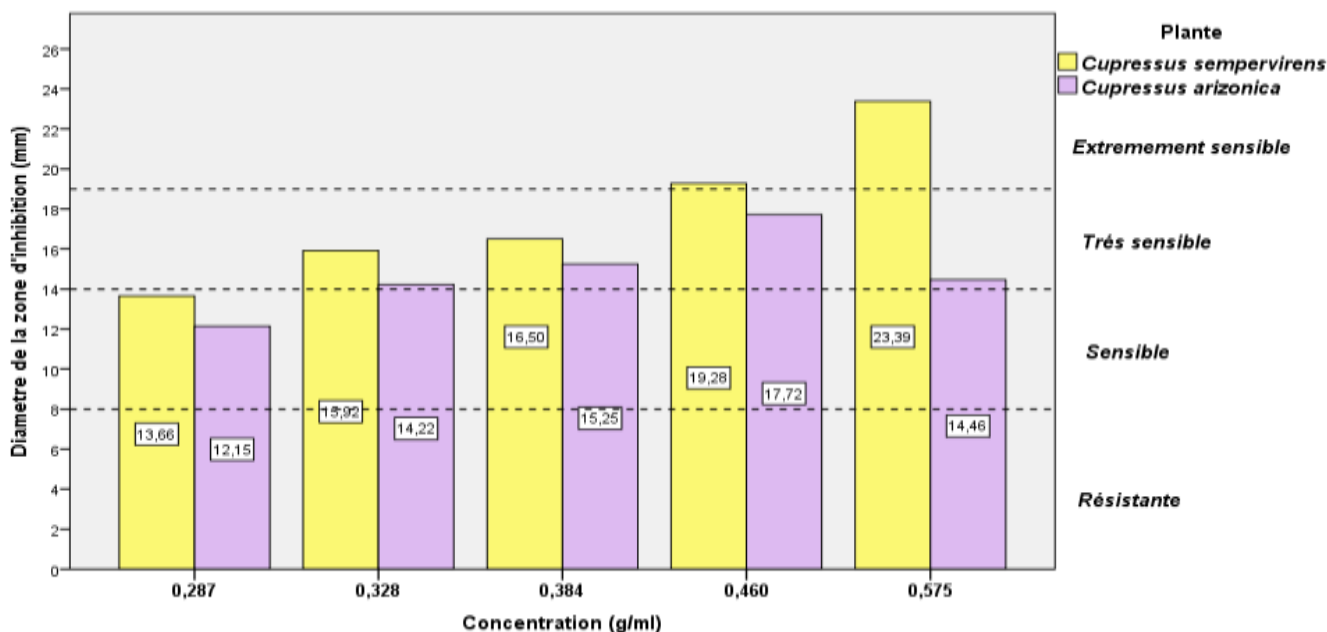


Figure 7: Effect of plant species and extract concentration on the inhibition zone diameter (mm) of *Pseudomonas savastanoi* pv. *Savastanoi*

Analysis of variance (ANOVA), followed by Tukey's post-hoc test, revealed significant differences between the antibacterial activities of the two hydroalcoholic extracts, indicating that *Cupressus sempervirens* exhibited greater inhibitory efficacy against *Pseudomonas savastanoi* pv. *savastanoi* than *Cupressus arizonica*. To further characterize this antibacterial response, the inhibition zone diameters (mm) produced by the different extract concentrations were measured and are presented in Figure 7. The observed variations in inhibition diameter provide additional evidence of the concentration-dependent sensitivity of *P. savastanoi* to both cypress extracts.

The susceptibility of *Pseudomonas savastanoi* pv. *savastanoi* increased progressively with increasing concentrations of both hydroalcoholic extracts, indicating a clear dose-dependent antibacterial effect. Larger inhibition zone diameters were consistently observed at higher extract concentrations, reflecting enhanced bacterial growth suppression. Notably, *P. savastanoi* exhibited an exceptionally high level of sensitivity to the *C. sempervirens* extract, which was classified as highly effective even at relatively low concentrations. The detailed classification of bacterial susceptibility to the phenolic extracts of *C. sempervirens* and *C. arizonica* throughout the experiment is summarized in Table 1.

Table 1: Susceptibility of *Pseudomonas savastanoi* pv. *savastanoi* to phenolic extracts of *Cupressus sempervirens* and *Cupressus arizonica*.

Plant Species	Concentration (g/mL)	Interpretation of the Inhibition Zone
<i>C. sempervirens</i>	0.287	Sensitive
	0.328	Highly sensitive
	0.384	Highly sensitive
	0.460	Extremely sensitive
	0.575	Extremely sensitive
<i>C. arizonica</i>	0.287	Sensitive
	0.328	Highly sensitive
	0.384	Highly sensitive
	0.460	Highly sensitive
	0.575	Highly sensitive

### 3.5. Lethal Dose Estimation

Probit regression yielded the following estimates:

**Table 2: Lethal dose estimates (LD<sub>50</sub> and LD<sub>99</sub>) obtained by Probit analysis for hydroalcoholic extracts of *Cupressus sempervirens* and *Cupressus arizonica* against *Pseudomonas savastanoi* pv. *savastanoi***

Species	LD <sub>50</sub> (g mL <sup>-1</sup> )	LD <sub>99</sub> (g mL <sup>-1</sup> )
<i>C. sempervirens</i>	3.44	62.72
<i>C. arizonica</i>	1.88	14.15

The substantially lower LD values recorded for *C. arizonica* indicate that this species achieves bacterial inhibition at considerably lower extract concentrations ; a finding with direct implications for practical formulation.

By integrating the extraction yield data with the estimated LD<sub>50</sub> and LD<sub>99</sub> values, it was possible to determine the amount of dry plant material required to achieve 50% and 99% inhibition of *Pseudomonas savastanoi* pv. *savastanoi*. The results presented in Table 2 indicate that, from an economic and practical perspective, *Cupressus arizonica* represents the most efficient species in terms of plant biomass utilization. Despite its lower extraction yield, its greater antibacterial efficacy allows the desired inhibitory effect to be achieved with a comparatively lower amount of plant material, highlighting its potential as a cost-effective source for the development of botanical antibacterial formulations.

**Table 3: Comparative assessment of dry biomass requirements for achieving LD<sub>50</sub> and LD<sub>99</sub> values of hydroalcoholic extracts from *Cupressus sempervirens* and *Cupressus arizonica* against *Pseudomonas savastanoi* pv. *savastanoi*.**

Hydroalcoholic Extract	LD <sub>50</sub> (g/mL)	LD <sub>99</sub> (g/mL)	Extraction Yield (%)	Dry Matter Required for LD <sub>50</sub> (g)	Dry Matter Required for LD <sub>99</sub> (g)
<i>C. sempervirens</i>	3.44	62.72	15.34	22.43	279.69
<i>C. arizonica</i>	1.88	14.15	11.31	16.62	85.13

**Abbreviations:** LD<sub>50</sub>, lethal dose required to inhibit 50% of the bacterial population; LD<sub>99</sub>, lethal dose required to inhibit 99% of the bacterial population; Dry Matter (DM), estimated amount of plant dry biomass required to obtain the corresponding lethal dose based on extraction yield.

LD<sub>50</sub> and LD<sub>99</sub> values were estimated using Probit regression analysis. Dry matter requirements were calculated by integrating extraction yield with the corresponding lethal dose values to estimate the quantity of plant biomass necessary to achieve bacterial inhibition.

## 4. DISCUSSION

The extraction yields we obtained confirm that hydroalcoholic solvents are effective for recovering bioactive compounds from cypress foliage. The higher yield from *C. sempervirens* aligns with what one might expect from a species adapted to Mediterranean conditions, where selective pressure may favor greater accumulation of soluble secondary metabolites.

The phenolic content detected in both species is consistent with earlier reports on the Cupressaceae. Phenolic compounds are among the most versatile weapons in a plant's chemical arsenal – they defend against herbivores, pathogens, and oxidative stress, and their presence in these extracts almost certainly underpins much of the biological activity we observed.

The inverted DPPH response deserves a closer look. It is unusual to see stronger radical scavenging at lower concentrations, and this pattern should not be dismissed as an experimental artifact. Several explanations are plausible. At high concentrations, polyphenols and other macromolecules may aggregate, reducing the

effective number of radical-binding sites. Alternatively, pro-oxidant behavior – a well-documented phenomenon for certain polyphenols at elevated concentrations – could shift the net redox balance. Whatever the mechanism, this finding underscores a broader point about working with complex mixtures: the dose-response relationship is not always linear, and assumptions about "more is better" do not always hold.

Turning to the antibacterial results, the data leave little doubt that both cypress extracts contain molecules capable of interfering with *P. savastanoi* pv. *savastanoi*. Phenolic compounds are known to disrupt bacterial membranes, alter cell permeability, denature structural and enzymatic proteins, and block essential metabolic pathways. Given the diversity of phenolics present in these extracts, the antibacterial effect is likely the product of multiple concurrent mechanisms – a feature that is particularly attractive from a resistance-management perspective, since multi-target inhibitors are less prone to selecting for resistant mutants.

The most practically relevant finding may be the contrast between the two species. *C. sempervirens* extracts more mass, but *C. arizonica* hits harder per unit mass. The LD<sub>50</sub> value for *C. arizonica* is roughly half that of *C. sempervirens*, and the LD<sub>99</sub> difference is even more striking – more than four-fold lower. This means that, pound for pound, *C. arizonica* leaf material would go further in a formulated product. When extraction yield and biological potency are weighed together, *C. arizonica* emerges as the more economically promising candidate.

Of course, laboratory data only take us so far. Field conditions introduce variables that cannot be replicated on agar plates – UV exposure, rainfall, leaf surface microbiology, and the complex biochemistry of the host plant itself. Still, these results provide a solid foundation for the next phase of work: formulation testing, in planta validation, and ultimately, field trials. The efficiency of plant extraction largely depends on the extraction solvent and its ability to recover bioactive compounds while preserving their chemical integrity. Organic solvent extraction aims to exhaustively recover extractable metabolites from plant tissues, followed by solvent removal through evaporation. The selection of an appropriate solvent is therefore a critical step in phytochemical investigations. According to **Turkmen and al. (2007)**, an effective extraction procedure should maximize the recovery of target compounds while minimizing chemical degradation and structural modifications during the extraction process.

In the present study, a hydroalcoholic methanol-water mixture was selected based on the phytochemical screening previously conducted on *Cupressus arizonica* and *Cupressus sempervirens* by **Degaïchia and al. (2022)**. Their findings demonstrated that hydroalcoholic extraction was particularly effective for recovering phenolic compounds, which were found to be abundant in the leaves of both species. The use of a methanol-water system has been reported to improve the extraction efficiency of phenolic constituents due to its ability to dissolve a broad spectrum of polar and moderately polar metabolites.

Similarly, **Rahmani (2020)** investigated the distribution of phenolic compounds among different organs of *Cupressus* species and reported that phenolic content increased with solvent polarity. Furthermore, among the various plant organs examined, leaves exhibited the highest concentration of phenolic compounds. In agreement with these observations, the hydroalcoholic extracts obtained from the leaf scales of *C. sempervirens* and *C. arizonica* in the present study yielded appreciable amounts of phenolic constituents, confirming the suitability of these plant materials as sources of bioactive compounds.

The findings of **Gouizi and Shoutri (2020)** further support our results. These authors reported the presence of significant amounts of phenolic compounds in methanolic extracts of *Cupressus sempervirens*, with total phenolic contents reaching 8.3 mg gallic acid equivalents (GAE) per gram of extract. Their results corroborate earlier reports published by **Bouzari and Belkram (2019)**, **Yahiaoui (2019)**, **Chihat et al. (2025)** and **CHADI et al. (2025)**, which highlighted the richness of cypress species in phenolic metabolites and their associated biological activities. The antioxidant evaluation conducted in the present study revealed an inverse relationship between extract concentration and radical scavenging activity. More specifically, antioxidant activity increased as extract concentration decreased. At first glance, this behavior may appear counterintuitive; however, several mechanisms may explain such a response.

First, biological and chemical systems do not always exhibit a linear dose-response relationship. In many cases, antioxidant activity follows U-shaped or J-shaped patterns, where maximum activity is observed within a specific concentration range before declining at higher concentrations. Under such conditions, low concentrations may favor optimal interactions between antioxidant molecules and free radicals.

Second, plant extracts are highly complex mixtures composed of numerous bioactive molecules. At elevated concentrations, interactions among these compounds may become antagonistic, potentially reducing the overall antioxidant efficiency. Certain constituents may interfere with the activity of others, thereby limiting their radical-scavenging capacity.

Third, different antioxidant mechanisms may predominate depending on extract concentration. Some compounds may be highly effective hydrogen or electron donors at lower concentrations, whereas other constituents may become more influential at higher concentrations. The resulting balance among these mechanisms can significantly affect the overall antioxidant response.

Finally, the chemical reactivity of antioxidant molecules may vary according to concentration. Complex redox reactions, molecular aggregation phenomena, and changes in compound stability may alter the effectiveness of antioxidant constituents under different experimental conditions.

Comparable observations were reported by **Gouizi and Shoutri (2020)**, who suggested that the antioxidant activity of *Cupressus* extracts cannot be explained solely by total phenolic content. Although relatively low concentrations of phenolic compounds were detected, the extracts exhibited substantial antioxidant activity. This finding suggests that either highly active phenolic molecules are present or that non-phenolic constituents contribute significantly to the antioxidant potential of the extracts.

**Ait Ialeff (2022)** also reported significant antioxidant activity in *Cupressus sempervirens* extracts at low concentrations. Our results are consistent with these findings and further demonstrate that both *C. sempervirens* and *C. arizonica* exhibit concentration-dependent antioxidant behavior, characterized by decreasing radical-scavenging activity as extract concentration increases.

More recently, **Boucif and al. (2023)** reported that all organs of cypress species possess remarkable antioxidant potential. Their findings highlighted the importance of *Cupressus* species as natural sources of antioxidant compounds and emphasized their potential applications in pharmaceutical, food, and agricultural sectors.

The antibacterial activity observed in the present study is most likely associated with the chemical composition of the extracts. Differences in antimicrobial efficacy between the two species may reflect variations in the qualitative and quantitative composition of their bioactive metabolites. According to **Machiex and al. (2005)**, the antimicrobial properties of plant extracts are strongly influenced by the nature and concentration of active compounds present within the extract.

Statistical analysis of the antibacterial assays demonstrated that the inhibitory effect of both extracts against *Pseudomonas savastanoi* pv. *savastanoi* was concentration dependent. The hydroalcoholic extract of *C. sempervirens* generally exhibited stronger antibacterial activity than that of *C. arizonica*. These observations are consistent with the phytochemical screening conducted by **Degaïchia and al. (2022)**, which revealed differences in the composition and abundance of secondary metabolites according to plant species and extraction solvent.

The susceptibility of *Pseudomonas savastanoi* to antimicrobial agents has previously been documented. **Bensaber and al. (2022)** demonstrated that this phytopathogenic bacterium is sensitive to several conventional antibiotics, including amikacin, gentamicin, colistin sulfate, ciprofloxacin, cefotaxime, and fosfomycin. The present study extends these observations by showing that *P. savastanoi* is also susceptible to hydroalcoholic extracts of *C. sempervirens* and *C. arizonica*, with measurable antibacterial activity observed from the minimum tested concentration of 0.287 g mL<sup>-1</sup>. These findings reinforce the potential of cypress-derived extracts as promising natural antibacterial agents for the management of olive knot disease.

## 5. CONCLUSION

This study demonstrates that hydroalcoholic extracts from *C. sempervirens* and *C. arizonica* carry substantial phenolic loads and exhibit genuine antioxidant and antibacterial activity against the olive knot pathogen. *C. sempervirens* gives the better extraction yield, but *C. arizonica* delivers superior antibacterial efficiency – a trade-off that tilts in favor of the latter when the goal is economical biopesticide production.

Both species merit further investigation. The next logical steps include comprehensive phytochemical profiling by HPLC-MS/MS and GC-MS, the development of stable formulations suitable for field application, and in planta testing under both controlled and open-field conditions. Toxicological and environmental safety assessments will also be necessary before any commercial development can proceed.

What is already clear is that these cypress extracts offer a genuine alternative to the copper-based treatments that have dominated olive disease management for decades. In an era of tightening pesticide regulations and rising resistance, that is a finding worth pursuing.

### Acknowledgments

The authors thank Dr. Hoceme Degaïchia (Research Center for Agropastoralism, Djelfa, Algeria) for botanical identification and Dr. Dounia Sadak (National Institute of Plant Protection, INPV, Boufarik Station, Algeria) for bacterial strain isolation and identification.

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