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Original Research Article

Phenolic Content and Antibacterial Activity of *Cupressus sempervirens* Leaves Extracts Against Phytopathogenic Bacteria *Pectobacterium atrosepticum* Causative Agent of Soft Rot on Potato and Clinical Bacteria Strains

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ABSTRACT

Cupressus sempervirens (Cupressaceae) "cypress" is a specie native to the Mediterranean region. It is a medicinal and an aromatic plant. This study was carried out to evaluate the antibacterial activity of Algerian *Cupressus sempervirens* leaves extracts against a phytopathogenic bacteria, *Pectobacterium atrosepticum*, a causative agent of soft rot on potato *Solanum tuberosum*, and three clinical bacterial strains *(Staphylococcus aureus, Escherichia coli and Klebsiella oxytoca*), in both cultured media and on potato tubers. Additionally, the Ethanolic, Acetone, and aqueous extracts were investigated for their phenolic content by using the Folin–Ciocalteu assay. The results revealed that all extracts possessed varying antibacterial activities against the tested Gram-positive and Gram-negative bacteria. *Pectobacterium atrosepticum* was the most susceptible microorganism to all extracts. Similar result of the antibacterial activity of the extracts, using extracts on potato tubers, was obtained. A decrease in soft rot development with almost complete inhibition in presence of extracts was observed. The total phenolic content of the investigated extracts varied from 8.30 ± 0.07 mg GAE / g extract to 27.68 ± 1.20 mg GAE / g of extract. The aqueous extract was the richest in total polyphenols (27.68 ± 1.20mg GAE / g of extract). From the results of this study, it is concluded that the Algerian cypress can be used as an effective natural antibacterial for treating multiple diseases, as well as an excellent biological control against the pathogens of potato rots, and natural food preservative.

Keywords: Phenolic compounds, Antibacterial activity, Biological control, *Pectobacterium atrosepticum, Solanum tuberosum L.*

Introduction

Potato (*Solanum tuberosum, L*) in Algeria represents the first vegetable crop in terms of production and consumption. Algeria is the second-largest potato producer in Africa, with a total potato production of 4,606,400.00 tons from 148,692.00 hectares. Algerian consumes 111 kg/person/year of potato, three times higher than the international average, estimated at 31 kg/person/year.[1] The production of potatoes is accompanied by severe diseases caused by bacterial phytopathogens, leading to enormous losses in yield and quality worldwide.[2] The major source of the soft rot bacteria is contamination by one or a combination of soft rotting pectinolytic enterobacteria.[3] This group has undergone considerable taxonomic revisions over the past 20 years, particularly, since the development of phylogenomic classification systems.[4] Previously, they were classified within the genus Erwinia, which was divided into several species and subspecies on the basis of molecular, biochemical and host range differences.[5]

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Pectobacterium atrosepticum classified in the *Enterobacteriaceae* family, is the most common cause of the soft rot disease of potato tubers in stores and in the field where early decay of mother tuber or seed tuber pieces may occur.[6] The cells of this species are usually rod-shaped Gram-negative bacteria with a pink color. They are characterized by the production of extracellular pectinolytic enzymes during the infection of plants.[2]

Soft rot bacteria cause extensive economic losses in potato production due to complexity of the pathogens, ubiquity, virulence, variation in symptom expression and their ability to multiply fast. Recently, controlling these bacterial plant diseases remains a challenge, as direct chemical control is usually not possible. Because of the high risk of the residual effect of toxic chemicals (such as sodium hypochlorite and the Nickel) that might be hazardous to consumers' health.[7] Obtaining new bio-control methods, specifically effective biomolecules like plant extract, able to boost defense reactions and limit infection or symptom development, is therefore of major importance.[8]

The evergreen cypress (*Cupressus sempervirens L.*) has been historically used for its therapeutic and medicinal properties.[9] Its natural distribution comprises the Aegean islands (Greece), Cyprus, Turkey (South Anatolia), North-east Africa (Libya, Algeria and Tunisia), and the Middle East (Iran, Jordan, Lebanon and Syria).[10] It is a slender tree that can grow up to 25 m in height. Unlike other plants, whose wood and roots are used in herbal medicine, in the case of Cypress, only the upper parts of the plant are used. The leaves are small and thin, they are used in the extraction of essential oil and natural extracts. The galbule or Cypress nut is the cone formed by the

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female flowers (Figure 1) which, earlier, are grouped together in the form of catkins. The seed cones are ovoid or oblong, 25–40 mm long, with 10-14 scales, green at first, maturing to brown about 20–24 months after pollination.

Several studies have reported the antiseptic, astringent, antispasmodic, vasoconstrictive, anti-inflammatory, antioxidant, antimicrobial, and antiviral activities of its extracts and essential oils, thus, rendering Cypress as a potential source of new drugs and natural antimicrobial compounds**.** [11]

Recently, plant sources have received a great deal of attention as a novel therapeutic alternative. Furthermore, they are abundant in active compounds that have antimicrobial activity and may substitute chemical-based food preservative. Although there are many reports available on the antimicrobial and antioxidant properties of plants extracts, there are relatively very few reports on their ability to mitigate the severity of soft rot. Considering the above facts, we extensively searched for an effective antibacterial agent for the most common potato soft rot strain; *Pectobacterium atrosepticum* and compare this effect with the inhibitory effect of these extracts against clinical strains bacteria (*Staphylococcus aureus, Escherichia coli* and *Klebsiella oxytoca),* the most isolated bacterial strains during nosocomial infections. The aim of this study was to evaluate the antibacterial and antioxidant activities and to investigate the effectiveness of green cypress (*Cupressus sempervirens* L.) extracts in inhibiting the growth of phytopathogenic bacteria *Pectobacterium atrosepticum* causative agent of soft rot on potato.

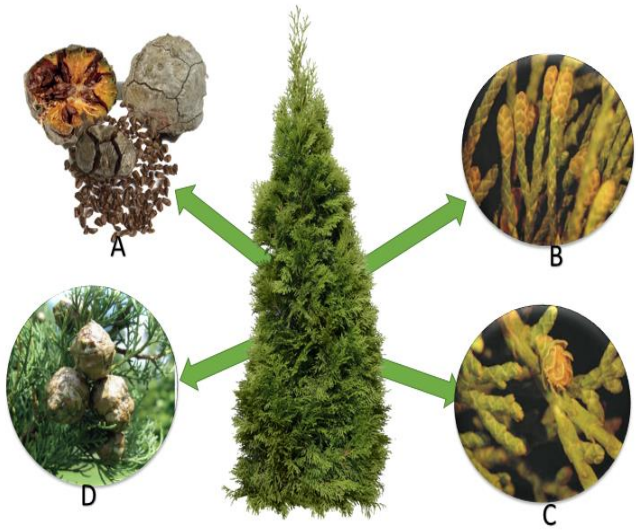

Figure 1: Cypress *Cupressus sempervirens L. (*A): Seeds, (B): flowers, (C): female flowers, (D): Cones

Materials and Methods

Plant material

Leaves (the aerial parts) of *Cupressus sempervirens* L., were collected from the Experimental Station at Saad Dahlab Blida 1 University situated in the center of Algeria in March 2020. The plants were identified by Pr Benrima Atika, from Laboratory Biotechnology of Plant Productions, Faculty of Nature and Life Sciences, Saad Dahlab University, Blida 1, where voucher specimens of *C. sempervirens* var. *horizontalis,* were deposited with voucher number of 157-0307. The plant material was air dried in shade at room temperature. The leaf petioles were carefully manually separated and dry leaves were crushed (3x1 min in high-speed grinder) into powder and stored at 4ºC protected from light until further use.

Sample preparation

For the extraction, a fine dried powder (50 g) of sample was added to 500 mL of distilled water, ethanol, methanol or acetone, homogenized and shaken at 150 rpm for 24 h, at room temperature. The extracts were filtered through Whatman No.1 filter. The residue was then extracted with two additional 100 mL portions of the same solvents (distilled water, ethanol, methanol or acetone). The combined extracts were evaporated by using a rotary evaporator or freeze dryer to give the crude dried extract. The resulting extracts were then dissolved in dimethlysulfoxide (DMSO) and kept at - 4°C until further use. [12-13]

Calculation of yield

The yield is the amount of extract obtained from the vegetable powder. It is expressed as a percentage from plant weight. In practice, we made the ratio of the mass of the extract to the mass of the vegetable powder which was multiplied by 100. This results in the formula given by Harbone.[14]

$$R = \frac{(Mass\ of\ obtained\ extract)}{(Test\ sample)} \times 100$$

Total phenolic content

The Folin–Ciocalteu method was used to determine the total phenolic content of the extracts according to Singleton *et al.*[15] with some modifications. The sample (2.5 mL) was diluted to 25 mL with distilled water. An aliquot of the solution (2 mL) was mixed with Folin–Ciocalteu reagent (10-fold diluted with distilled water, 10 mL). After 5 min, a 7.5% (w/v) sodium carbonate solution (8 mL) was added. After 2 h, the absorbance was measured at 765 nm against a blank prepared as described above with distilled water (2 mL), Folin–Ciocalteu reagent and sodium carbonate solution. Values of total phenolic content were estimated by comparing the absorbance of each sample with a standard response curve generated using gallic acid. The concentration of polyphenols present in the plant extracts expressed as mg gallic acid equivalents (GAE)/ g of extract can be calculated by the following formula:

$$[Polyphenols] = \frac{a\,.f}{b}$$

a: concentration of polyphenols in µg / mL determined from the standard curve.
f: dilution factor (x10)
b: initial concentration of extracts.

Potato tuber as a source of pathogens

The potato tubers were used for direct isolation of *Pectobacterium atrosepticum* strains. Infected samples of potatoes showing the characteristic symptoms of soft rot were taken from storage area in Kolea located in the South-west of Algiers (Algeria). Samples were brought into the laboratory in polythene bags.

Isolation and purification of Pectobacterium atrosepticum

Infected samples were cut into small pieces of 2 - 3 cm length and their surfaces sterilized with 1% $HgCl_2$ for 2 - 3 minutes with three successive washings in distilled water. The experimental protocol followed is detailed in Figure 2. Purification of bacterial colonies was done by re-streaking of a single colony on sterile nutrient agar plates and a suspension of 10^7 cells/mL in phosphate buffer solution pH 7.4 of each isolate was stored at −20°C in 10% glycerol. Finally, these isolates were inoculated onto potato tubers to confirm pathogenicity.

Isolation of human pathogenic bacteria

Clinical pathogenic bacterial strains, were isolated from patients in different hospital wards of the Hospital of Kolea (situated in the center of Algeria).

Identification of the pathogenic organisms

Gram staining and biochemical assays were performed on microbes grown anaerobically at 28° and 37°C in tryptone soya broth (TSB) media supplemented with 5% NaCl. Catalase and oxidase activity, indole production, and acid production from lactose, maltose, and raffinose were determined as previously described.[16-17]

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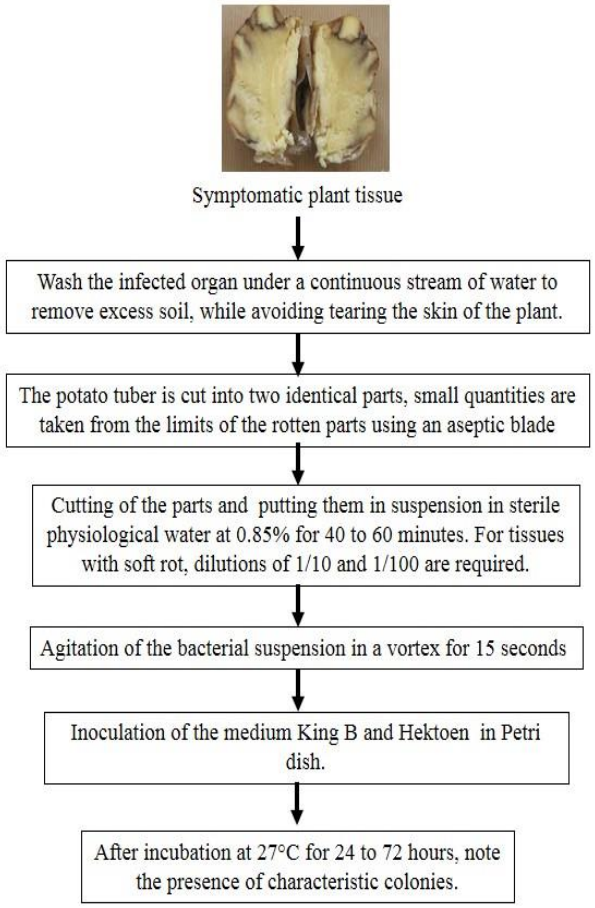

Figure 2: Isolation and Purification of *Pectobacterium atrosepticum.*

Confirmation of identification by MALDI-TOF mass spectrometry method

Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer is one of the most popular Mass Spectrometry instruments used in biology, due to its rapid and precise identification of genus and species of an extensive range of Gram-negative and Gram-positive bacteria. In this study, we confirmed the identification of the bacterial strains used by this rapid method. A colony was "picked" from a culture plate to a "spot" on a MALDI-TOF–MS target plate Microflex LT BIOTYPER (BRUKER)® (BrukerDaltonics, Germany). The addition of 1µL of matrix solution (10 mg/mL α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) to the MALDI plate was used to improve the quality of the generated mass spectrum. After drying, the target plate was placed in the mass spectrometer's ionization chamber. All spectra were compared with reference spectra of the BDAL database and with main spectrum profiles created. All identifications were reported with the following score values: < 1.7 was interpreted as an unreliable identification; 1.7–2.0 as a probable genus identification; 2.0–2.3 as a secure genus identification and probable species identification; and >2.3 was regarded as a highly probable species identification. Only the highest score value of all mass spectra belonging to individual cultures (biological and technical replicates) was recorded.

Pathogenicity tests on potato tubers

Potato tubers (*Solanum tuberosum*, L) were selected free of wounds, rots and homogeneous in size. They were washed, surface rinsed in sterile distilled water and air-dried under a laminar flow hood. Before inoculation, a bacterial suspension was prepared at 10^7cfu/mL concentrations in liquid nutritive media incubated at 28 °C for 24 h. The half-tuber method was used for the study of the pathogenic variability of *P. atrosepticum*.[18] Each tuber was cut in two roughly equal parts with a sterile knife. A hole (5 mm diameter - 5mm depth) was drilled with a cork borer in the center of each half-tuber. Ten half-tubers were inoculated by depositing 100µLof the bacterial suspensions prepared previously. Control tubers received 100µL of sterile distilled water. After 6 days incubation at 28°C in water-saturated environment, symptoms were assessed visually and the extent of soft rot was determined by weighing the rotted tissue for each tuber.

Determination of the antibacterial activity

Agar disc diffusion assay

Antibacterial activity was determined by the agar disc diffusion assay.[19] All the extracts were prepared by dissolving them in dimethyl sulfoxide (DMSO). Petri plates were prepared with 20 mL of sterile Mueller Hinton agar (Sigma, Paris, France). The surface of the medium was inoculated by suspension of cell (200 µL) adjusted by McFarland 0.5 method (10^6CFU/mL). Sterile filter paper discs of 6 mm diameter were impregnated with 20 µL of the extract solution. The plates were incubated at 28°C for 24 h. Negative controls were performed using paper discs loaded with 20 µL of the solvents used. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs. After that, the inhibition zones were measured in millimeters by Vernier calipers.

Antibacterial activity on potato tubers

The same technique as the pathogenicity test described above was used except that the tubers were inoculated by depositing 100µLof the bacterial suspensions with 100µL of *Cupressus sempervirens L.* leaves extracts. After 6 days incubation at 28°C in water-saturated environment, the rotted tissue was collected with a spatula and weighed.

Statistical data analysis

All the experiments were carried out in triplicate. The quantitative variables were expressed in number and percentage. Means and standard deviations were calculated with SPSS 20.0 software (Chicago, USA). A single factor analysis of variance (ANOVA) along with the post-hoc Tukey's test was employed for determine the difference between the variables. The variables were considered to indicate a statistically significant difference for a $p \leq 0.05$.

Results and Discussion

Taxonomic characterization of the selected isolates

Ten isolates of pectinolytic bacteria were isolated from infected potato tubers. Three of them were identified as *Pectobacterium atrosepticum* according to their morphological and biochemical characters (Table 1). The bacterium was rod shaped with small, irregular, rounded ends, convex, and creamy white colonies of cells appeared both singly and in pairs. The isolated bacteria were Gram-negative, facultative anaerobic. Additionally, these bacteria reacted positively to catalase and the production of reducing substances from sucrose, but reacted negatively to oxidase, the production of phosphatase, and indole. They did not grow at 37 °C but grew in TSB + 5% NaCl under anaerobic conditions and produced acid from lactose, maltose and raffinose. These tests enabled the strains to be classified as *P. atrosepticum* species. Three clinical isolated bacteria from hospitalized patients were identified according to their morphological and biochemical character; one Gram + strain (*Staphylococcus aureus*) and two Gram- strains (*Escherichia coli, Klebsiella oxytoca*), and confirmed by MALDI-OF-MS software**.**

Pathogenicity tests

A three tested bacterial isolates were pathogenic and produced soft rot symptoms on potato tubers. The *P. atrosepticum* isolate 3 showed a high disease index (86.04%). The other isolated strains caused no symptom when inoculated on potato tubers.

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Extraction yield and total phenolic content

The extraction of the phenolic compounds by solvents with different polarity from *Cupressus sempervirens L.* leaves studied, allowed us to determine the yields of their crude extracts (Figure 3). Through these obtained results, we note that the best yields were obtained by the aqueous extract (25%) and methanol extract (20.4%) followed by the acetone extract (14.96%) and the ethanol extract (11, 48%). These results are similar to the ones obtained by other studies done on *C. sempervirens*L. in Algeria.[20-23] However, this rate is higher than that reported by Ebrahim *et al.*[24], which is 6.09% by using chloroform as solvent. These differences obtained in the extraction yields for the same species are linked to several factors such as genetic and environmental factors, part of the plant, methods of isolation, harvesting time and geographical location.[24]

According to scientific literature, the best solvents are ethanol and methanol, and ultrasonic extraction generally improves extraction efficiency. Thongson *et al.*[25] reported that ultrasonic extraction method only took 5 minutes to obtain the bioactive components of a medicinal plant.[25] The total phenolic content for ethanol, acetone, and aqueous extracts were estimated by Folin Ciocalteu's method using gallic acid as standard.

Table 1: Morphological appearance and results of biochemical tests of the isolated strains

Test	**Isolate 1**	**Isolate 2**	**Isolate 3**
Morphological aspect	**rod shaped, convex, white colonies**	**rod shaped, convex, white colonies**	**rod shaped, convex, white colonies**
Gram stain	–	–	–
Growth in nutrient agar (24 h, at 28°C)	+	+	+
Growth in nutrient agar (24 h, at 37°C)	–	–	–
Growth in 5% NaCl	+	+	–/+
Oxydase	–	–	–
Catalase	+	+	+
Production of reducing substances from sucrose	+	+	+
Production of indole	–	–	–
Production of phosphatase	–	–	–
Acid production from lactose	+	+	+
Acid production from maltose	+	+	+
Acid production fromtrehalose	+	+	+
Acid production from sorbitol	–	–	–
Identification	*P. atrosepticum*	*P. atrosepticum*	*P.atrosepticum*

The results of this assay Figure 4, illustrated that the total phenolic content of the investigated extracts varied from 8.3 ± 0.07 mg GAE/g extract to 27.68 ± 1.20 mgGAE/g of extract. The aqueous extract was the richest in total polyphenols (27.68 ± 1.20 mg GAE/g of extract), followed by the ethanol extract with a content of 10.25 ± 0.6 mgGAE/g extract. On the other hand, low levels were recorded in the methanol extract and the acetone extracts (8.3 ± 0.07 mg EAG/g extract and 7.54 ± 0.06 mg GAE/g of extract) respectively. Our results are in disagreement with those reported by Aloui *et al.*[26] (138.67 ± 2.69 mg GAE/g) and Hammadache[20] (125.06 mg GAE/g) respectively. In the same study,[20] phenolic content of *C. superverens* leaves extract was the highest in comparison with other plants extracts; *Quercus ilex* (110.8 mg GAE/g of extract), *Ephedra altissima* (102.03 mg GAE/g of extract), *Marrubium vulgare* (46.06 mg GAE/g of extract) and *Mirabilis jalapa* (43.46 mg GAE/g of extract).

Antibacterial activity

The *in vitro* antimicrobial potential of *C. sempervirens* extracts against a phytopathogenic bacteria *Pectobacterium atrosepticum* and three clinical bacteria strains; *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella oxytoca*is are shown in Table 2. All extracts showed moderate *in vitro* antibacterial activity. In the comparison of bacterial sensitivity, *Pectobacterium atrosepticum* seem to be more sensitive to methanol and acetone extract than other pathogenic bacterial strains. No remarkable activity was observed for ethanol and acetone extracts against *E. coli*, which was revealed to be the most resistant strain. These findings are in accordance with recent published results for two Lebanese *Cupressus sempervirens* L. varieties (Beit El-Dein and Jbeil).[27] The results obtained in this study showed that the antibacterial activity of ethanol extract was more efficient against Gram positive than Gram-negative bacteria. The plant extracts inhibited the growth of the tested bacterial strains with an inhibition zone diameter ranging from 7-12 mm for Beit El-Dein samples and from 9.5-12.3 mm, for Jbeil samples. *S. aureus* showed the highest susceptibility with a larger diameter of inhibition zone (12-12.3 mm) for Beit El- Dein and Jbeil samples, respectively. On the other hand, *P. aeruginosa* showed the highest resistance with lowest diameters of inhibition zone (7 to 9.5 mm). According to the results of this study, ethanol extracts exhibited significantly less antibacterial activity compared to the essential oils previously reported.[18] However, the methanol extract of *C. sempervirens* L. plant leaves showed a moderate antibacterial activity but less as compared to the essential oil effect reported in a study carried out in Egypt.[28] Currently, the major objective of modern agriculture is to offer a strategy that would lead to minimizing the use of chemical pesticides, at the same time increasing the economic yield of crops. In addition, the fast development of phytopathogenic bacteria resistance to synthetic antibacterial agents makes it necessary to seek novelty and safe sources of molecules: wild plants have a great potential because they accumulate thousands of secondary metabolites.[29] To the best of our knowledge, no study has been done on the antibacterial activity of *Cupressus sempervirens* L. extracts against phytopathogenic bacteria *Pectobacterium atrosepticum* causative agent of soft rot on potato, the results obtained in this study revealed that Ethanol extract from *C. sempervirens* leaves reduced rot weight in potato tuber slices inoculated with *P. atrosepticum*, compared to water control treatments (Figure 5). The Aqueous extract from the same batch of leaves also tended to reduce rot weight, but its efficacy was less than that of ethanol extract. This observed effect could be attributed to the phenolic compounds. Indeed, plant based antimicrobial agents have been used as natural preservatives in foods for a long while. Although, the antimicrobial effect against pathogenic and saprophytic microorganisms was studied several times, the exact mechanism of inhibition has not been defined clearly. However, it is known, that flavonoids as well as phenolic acids are synthesized by

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plants in response to microbial infection.[30] Many reports have investigated the phenolic content of *C. sempervirens* and various classes have been revealed in leaves, including flavonoid compounds (rutin, quercetin, quercetin rhamnoside, quercitrin, myricitrin and kaempferol 3-*O*-rhamnoside, cupressuflavone, amentoflavone, and other biflavonoids),[31-32] catechins and flavonolic oligomers[33], proanthocyanidins[34] and phenolic acids (caffeic acid and p-coumaric acid).[32] The antibacterial activity of polyphenols has been widely studied. According, to Ashmawy *et al.*,[35] ferulic and tannic acids had a substantial inhibitory impact on the growth of *Pectobacterium carotovorum* isolates. A mixture of caffeic and chlorogenic acids could prevent bacterial soft rot infection from occurring, and the major phenolic acids detected in the tuber peels that had soft rot antimicrobial effects were chlorogenic, caffeic, and ferulic acids.[36]
The mechanism of the toxicity of polyphenols against microbes may be related to inhibition of hydrolytic enzymes (proteases) or other interactions that inactivate microbial adhesins, cell envelope transport proteins and non-specific interactions with carbohydrates.[37] It has been reported that the tannins were strong microbial inhibitors.[38] The toxicity of tannins may be largely due to the *O*-diphenol groups of tannins enabling them to act as iron chelators, thus depriving microorganisms of this essential element.[39] Moreover, the tannins have the ability to complex with extracellular and soluble proteins (such as the extracellular pectinolytic enzymes during the infection of plants) and also with bacterial cell walls.[40]

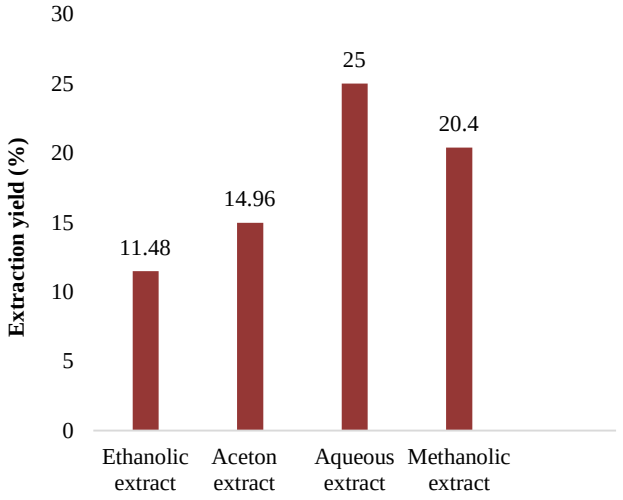

Figure 3: Results of extraction yields of *Cupressus sempervirens L* leaves.

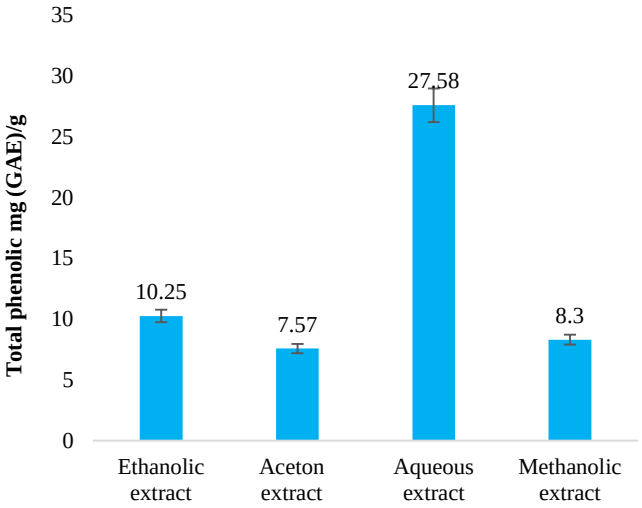

Figure 4: Total phenolic content as gallic acid equivalent (GAE mg/ g) in *Cupressus sempervirens* leaves extracts

Table 2: Antibacterial activity of the crude extracts of *Cupressus sempervirens l* leaves extracts in agar diffusion assay

Extracts	Mean Inhibition Zone Diameter (mm) for all tested bacterial strains			
	P. atrosepticum	***E.coli***	***S. aureus***	***K.oxytoca***
Ethanol	13.10	00	12.96	14.15
Methanol	13.90	13.76	12.29	7
Acetone	15.30	00	12.42	8.51
Aqueous	9.86	11.96	11	9.90

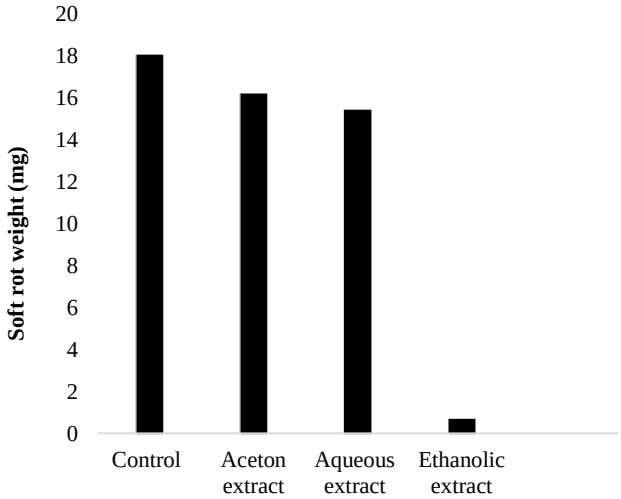

Figure 5: Effect of *Cupressus sempervirens* leaves extracts on soft rot development induced by *Pectobacterium atrosepticum* in potato slices

Conclusion

From the results of this study, which showed for the first time the effectiveness of the Algerian *C. sempervirens* L extracts against phytopathogenic bacteria *Pectobacterium atrosepticum* causative agent of soft rot on potato and human pathogenic bacteria, this effect is perhaps due to its high content of phenolic compounds. It can be concluded that the Algerian *C. sempervirens* L plant has promising potentials for beneficial health uses in treating and preventing many human diseases. Therefore, it can be used as a biological control against *Pectobacterium atrosepticum* causative agent of soft rot on potato plant and food preservation (as a natural alternative to chemicals), which may contribute positively to improving public health, providing the nutritional needs of society and individuals, increasing agricultural production and improving the economy outcomes. Additional *in-vitro*, *in-vivo* studies and clinical trials would be useful to justify and further evaluate the potential antimicrobial and antioxidant activities and to isolate, identify and elucidate the active compounds of this plant.

Conflict of interest

The authors declare no conflict of interest.

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Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. FAOSTAT, 2019. Agriculture data source. [Online]. 2019 [cited 2020 Dec 20] (Online) Available from:URLhttp://faostat.fao.org/default.aspx.
2. Pérombelon MCM. Potato diseases caused by soft rot erwinias: An overview of pathogenesis. Plant Pathol. 2002; 51:1-12.
3. Charkowski A, Blanco C, Condemine G, Franza T, Hayes C,Hugouvieux-Cotte-Pattat N, Solanilla EL,Low D, Moleleki L,Pirhonen M, Pitman A, Perna N, Reverchon S, Palenzuela PR, Francisco MS, Toth I, Tsuyumu S, Waals JVD, Wolf JVD, Gijsegem FV, Yang CH, Yedidia I. The role of secretion systems and small molecules in soft-rot *Enterbacteriaceae* pathogenicity. Annu Rev Phytopathol. 2012; 50:425-429.
4. Adeolu M, Alnajar S, Naushad S, Gupta RS. Genome-based phylogeny and taxonomy of the "Enterobacteriales": proposal for Enterobacterales ord. nov.divided into the families *Enterbacteriaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. Int J Syst Evol Microbiol. 2016; 66:5575-5599.
5. Beattie GA. Plant-associated bacteria: survey, molecular phylogeny, genomics and recent advances. In: Gnanamanickam S.S. (eds) Plant-Associated Bacteria. Springer, Dordrecht; 2007; 25-30p.
6. Gardan L, Gouy C, Christen R, Samson R. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov., and *Pectobacterium wasabiae* sp. nov. Int J Syst Evol Microbiol. 2003; 53:381-391.
7. Remor AP, Totti CC, Moreira DA, Dutra GP, Heuser VD, Boeira JM. Occupational exposure of farm workers to pesticides: biochemical parameters and evaluation of genotoxicity. Environ Int. 2009; 35:273-278.
8. Jayaraj R, Megha P, Sreedev P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. InterdiscipToxicol. 2016; 9(4):90-100.
9. Hussain MK, Saquib M, Ahamad T, Khatoon S, Khan MF. Mediterranean Cypress "*Cupressus sempervirens*": A Review on phytochemical and pharmacological properties. Curr Tradit Med. 2019; 5(4):278-297.
10. López-Sáez JA, Glais A, Tsiripidis I, Tsiftsis S, Sánchez-Mata D, Lespez L. Phytosociological and ecological discrimination of Mediterranean cypress (*Cupressus sempervirens*) communities in Crete (Greece) by means of pollen analysis. Mediterr Bot. 2019; 40(2):145-163.
11. Orhan IE and Tumen I. Potential of *Cupressus sempervirens* (Mediterranean Cypress) in Health. In: VR Preedy, RR Watson (Eds). Mediterranean Diet: An Evidence-Based Approach. Londres: Academic Press; 2015; 639-648p.
12. Debib A and Boukhatem MN. Phenolic content, antioxidant and antimicrobial activities of "Chemlali" olive leaf (*Olea europaea* L.) extracts. Int J Pharmacol Phytochem Ethnomed. 2017; 6:38-46.
13. Sahnouni F, Debib A, Saim S, Bouhadi B, Menadi S. Phytochemical Content, Antioxidant and Antibacterial Activities of Three Red Macroalgae from Algerian West Coast. Trop J Nat Prod Res. 2021; 5(2):336-341.
14. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. (3rd Ed). London (U.K.): Chapman & Hall; 1998; 235p.
15. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. Method Enzymol. 1999; 299:152-178.
16. Charkowski AO. Biology and control of Pectobacterium in potato. Am J Potato Res. 2015; 2(92):223-229.
17. Smolarska A, Rabalski L, Narajczyk M, Czajkowski R. Isolation and phenotypic and morphological characterization of the first *Podoviridae* lytic bacteriophages ϕA38 and ϕA41 infecting *Pectobacterium parmentieri* (former *Pectobacterium wasabiae*). Eur J Plant Pathol. 2018; 150:413-425.
18. Meziani S, Oomah BD, Zaidi F, Simon-Levert A, Bertrand C, Zaidi-Yahiaoui R. Antibacterial activity of carob (*Ceratonia siliqua* L.) extracts against phytopathogenic bacteria *Pectobacterium atrosepticum*. Microb Pathog. 2015; 78:95-102.
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 13th edition. CLSI document M02.2018. Wayne, PA: Clinical and Laboratory Standards Institute. 2018; 30-60p.
20. Hammadache N. Screening of phenolic extracts of plant origin endowed with antibacterial activity: search for natural β-lactamase inhibitors. Magister's thesis. Abderrahman Mira of Bejaia University. Algeria. [Online]. 2011. [cited 2020 Dec 1]. Available from: https://www.pnst.cerist.dz/detail.php?id=59041.
21. Mazari K, Bendimerad N, Bekhechi C, Fernandez X. Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens*L. J Med Plants Res. 2010; 4(10):959-964.
22. Fadel H, Benayache F, Chalchat JC, Figueredo G, Chalard P, Hazmoune H, Benayache S. Essential oil constituents of Juniperus oxycedrus L. and Cupressus sempervirens L. (Cupressaceae) growing in Aures region of Algeria. Nat Prod Res. 2019; 33:1-5.
23. Boudjemaa H. Antibacterial Activity of Ethyl Acetate Extracts from Algerian *Cupressus sempervirens* var Against Some Human Pathogens Bacteria. Algerian Journal of Natural Products. 2017; 5(3):524-529.
24. Ibrahim NA, El-Seedi HR, Mohammed M. Constituents and biological activity of the chloroform extract and essential oil of *Cupressus sempervirens*. Chem Nat Compd. 2009; 45(3):309-313.
25. Thongson C, Davidson PM, Mahakarnchanakul W, Weiss J. Antimicrobial activity of ultrasound-assisted solvent-extracted spices. Lett Appl Microbiol. 2004; 39(5):401-406.
26. Aloui F, Nefzi A, Jedidi S, Selmi H, Hasnaoui F, Dallali S, Bouraoui H, Mouhbi R, Abbes C. Phytochemical analysis and *in vitro* digestibility evaluation of leaf and cones parts of *Cupressus sempervirens* originating from Tunisia. J New Sci Agric Biotech. 2020; 71(2):4311-4318.
27. Anka L, Rammal H, Kobeissi A, Saab HB. Chemical composition and biological potentials of Lebanese *Cupressus sempervirens* L. leaves' extracts. J Med Plants Res. 2020; 14(6):292-299.
28. Selim SA, Adam ME, Hassan SM, Albalawi AR. Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean Cypress (*Cupressus sempervirens*L.). BMC Compl Altern Med. 2014; 14(1):1-8.

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29. Wianowska D, Garbaczewska S, Cieniecka-Roslonkiewicz A, Dawidowicz AL, Jankowska A. Comparison of antifungal activity of extracts from different *Juglans regia* cultivars and juglone. Microb Pathog. 2016; 100:263-267.
30. Debib A, Tir-touil A, Mothana RA, Meddah B, Sonnet P. Phenolic content, antioxidant and antimicrobial activities of two fruit varieties of Algerian *Ficus carica L.*J Food Biochem. 2014; 38:207-215.
31. Romani A, Galardi C, Pinelli P, Mulinacci N, Heimler D. HPLC quantification of flavonoids and biflavonoids in *Cupressaceae* leaves. Chromatographia 2002; 56:469-474.
32. Ibrahim NA, El-Seedi HR, Mohammed MMD. Phytochemical investigation and hepatoprotective activity of *Cupressus sempervirens* L. leaves growing in Egypt. Nat Prod Res 2007; 21:857-66.
33. Jonadet M, Meunier MT, Villie F, Bastide J, Bastide P. Catechins and flavanolic oligomers from *Cupressus sempervirens* L. Comparative in vitro elastase inhibitory and in vivo angioprotective activity. Ann Pharm Fr. 1984; 42:161-167.
34. Vennat B, Gross D, Pourrat A, Pourrat H. Polyphenols from *Cupressus sempervirens*: Qualitative and quantitative assays of proanthocyanidins and flavonoids. Farmaco 1991; 46:685-698.
35. Ashmawy NA, Behiry SI, Al-Huqail AA, Ali HM, Salem MZM. Bioactivity of Selected Phenolic Acids and Hexane Extracts from *Bougainvilla spectabilis* and *Citharexylum spinosum* on the Growth of *Pectobacterium carotovorum* and *Dickeya solani* Bacteria: An Opportunity to Save the Environment. Processes. 2020; 8(4):482.
36. Joshi JR, Burdman S, Lipsky A, Yariv S, Yedidia I. Plant phenolic acids affect the virulence of *Pectobacterium aroidearum* and *P. carotovorum* ssp. *brasiliense* via quorum sensing regulation. Mol Plant Pathol. 2015; 17:487-500.
37. Pyla R, Kim TJ, Silva JL, Jung YS. Enhanced antimicrobial activity of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant. Int J Food Microbiol. 2010; 28:154-160.
38. Kivçak B, Mert T, Ozturk HT. Antimicrobial and cytotoxic activity of *Ceratonia siliqua* L. Extracts. Turk J Biol. 2002; 26:197-200.
39. Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991; 12:3875-3883.
40. AL-Younis NK and Awaz FA. Isolation and antibacterial evaluation of plant extracts from some medicinal plant in Kurdistan region. J Duhok Univ. 2009; 12:250-255.

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