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ASSESSMENT OF PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *EVERNIA PRUNASTRI* SPECIES COLLECTED FROM ALGERIA

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ABSTRACT

In this study, the phytochemical screening of lichen samples of *Evernia prunastri* collected from three different regions of Algeria (Jijel sample (JS), Setif sample (SS) and M'sila sample (MS)) was investigated. The phenolic and flavonoid contents of lichen extracts obtained by maceration in methanol were determined. The antioxidant activities of these extracts were measured by determining the total antioxidant capacity (TAC), the ferric reducing antioxidant power (FRAP) and the free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Similarly, antibacterial activity was determined by solid medium diffusion and liquid medium microdilution methods against Gram-positive and Gram-negative bacteria. The qualitative phytochemical analysis of lichen samples revealed the presence of some compounds such as saponins, flavonoids, alkaloids. For quantitative analysis, the MS methanolic extract from M'Sila region showed the highest values for phenolic and flavonoid compounds which are equal to 929.3 mg/100g and 56.34 mg/100g, respectively. This extract also shows the best antioxidant activities with the three tested methods. For antibacterial activity, the best effect was obtained for the methanolic extract from Jijel region (JS) against the methicillin resistant *Staphylococcus aureus* strain with an inhibition zone of 35.33 mm and an MIC of 0.058 mg/mL. However, the three *Evernia prunastri* methanolic extracts (JS, SS and MS) were found to be inactive against Gram-negative bacteria. The obtained results indicate that the studied extracts have interesting antioxidant and antibacterial activities, which is probably due to the presence of phenolic and flavonoid compounds.

1. Introduction

Lichens are cryptogams that are present in all terrestrial ecosystems, including extreme ecosystems. They represent unique life forms- a symbiosis between a fungus (mycobiont) and an alga and/or a cyanobacterium (photobiont). They typically grow on rocks and non-fertile soils and as epiphytes on trees and leaves (Stamenković *et al.,* 2011; Chahra *et al.,* 2016).

For centuries, lichens have been used in the nutrition of many animals and humans during famines. Many edible lichens species such as *Dermatocarpon miniatum*, *Lobaria pulmonaria*, *Umbilicaria esculenta*, *Alectoria asiatica* are used in China as dishes after a simple transformation. They are also used as

spices, dyes in textiles, odorant in the perfume industry and for medicinal purposes to treat kidney, respiratory and liver infections and also as antiseptic (Mitrović *et al.,* 2011; Aoussar *et al.,* 2021; Muthu *et al.,* 2021; Zhao *et al.,* 2021).

Lichens are considered as a potential source of new biologically active compounds. They produce specific chemicals, phenols, that are very different from those synthesized by the rest of the plant species: about 1050 compounds in all and more than 550 compounds that are specific to lichens. Lichenic substances include aliphatic, cycloaliphatic, aromatic and terpene components (Herrero-Yudego *et al.,* 1989; Buçukoglu *et al.,* 2013; Kalidoss *et al.,* 2020). These metabolites are key components in the bioactivity of lichen extracts that are of great importance to modern pharmacy and medicine. Various biological activities of some lichens are known, such as antimicrobial, antiviral, antitumor, anti-inflammatory, analgesic, antipyretic, antiproliferative and antiprotozoal activities (Kosanic *et al.,* 2011; Mitrović *et al.,* 2011). Lichens as valuable sources of natural antioxidants and antimicrobial agents have been extensively studied. A strong antioxidant power of some lichen species was demonstrated in several studies (Kosanic *et al.,* 2011; Mitrović *et al.,* 2014).

The increasing development of microorganism resistance to conventional antibiotics and the problem of treatment of induced infections have prompted researchers to find other antimicrobial alternatives in lichens. Antibacterial activity against Gram-positive and Gram-negative bacteria, as well as antifungal activity are demonstrated for many lichen species (Mitrović *et al.,* 2011), such as *Evernia prunastri* (L.) Ach., *Ramalina fastigiata* (Pers.) Ach. and *Cladonia rangiformis* Hoffm. collected in northeastern Algeria which showed a very interesting antibacterial activity against *Staphylococcus aureus* strain. This activity is certainly due to predominant compounds such as lichenic acids which have already proved their antibacterial efficacy (Brakni and Ali Ahmed, 2018). Usnic acid as a pure substance has been formulated in creams, toothpastes, mouthwashes, deodorants and cosmetics, sunscreen products (Behera *et al.,* 2005).

According to the study of Miara *et al.* (2013), some species including *Evernia prunastri* (L.) Ach., were proposed as new species for the Algerian medicinal flora. Indeed, these species do not appear in the main works on therapeutic plants in Algeria, including the works of Baba-Aissa (1999) and those of Beloued (2009). According to these authors, other studies should be undertaken in the future, especially the phytochemical and pharmacological aspects of these new species.

Thus, in this context we propose through this work for the investigation of new natural sources with potential therapeutic effects from a species belonging to the group of lichens which is *Evernia prunastri* (L.) Ach. To the best of our knowledge, no study has already considered the study of this Algerian species, especially the one collected from the three cited regions namely Jijel (North-East Algeria), Setif and M'Sila (Steppe of Algeria). The aim of this study is to investigate the qualitative and quantitative phytochemistry and to determine the antibacterial and antioxidant activities of these three samples of *Evernia prunastri*.

2. Materials and methods

2.1. Lichen collection and preparation

The thallus of three samples of the lichen JS, SS and MS were collected respectively in the regions of Texenna (Jijel, North-East Algeria), Aïn Lahdjar (Setif, Algerian Steppe) and BouSaada (M'Sila, Algerian Steppe).

The lichen material was identified by Dr. Samira Salem (Laboratory of biotechnology, environment and health, Jijel university, Algeria). The intact thalli of *Evernia prunastri* (Figure 1) were dried at 40 °C and ground in an electric grinder into a fine powder and passed through very fine porosity sieves (250 µm). It was then put into boxes and stored for later use.

Figure 1. *Evernia prunastri* (L.) Ach. sample

2.2. Phytochemical analysis

The lichen powder was tested for the presence of saponins, alkaloids, tannins, flavonoids and terpenoids. Qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

2.2.1. Phytochemical screening

Saponins test (Frothing test)

Saponins were identified according to the method described by Banso and Adeyemo (2006). For this purpose, an aliquot of 0.5 g of different lichen powders was mixed in a test tube containing 3 mL of hot distilled water (100°C), then the whole was shaken vigorously and continuously (1 min) to observe the persistence of a foam.

Flavonoids test (Cyanidine test)

This test was performed according to the method of Tiencheu *et al.* (2021). An aliquot of 0.5 g of each lichen powder was mixed with 2 mL of methanol, and then 1mL of concentrated sulfuric acid was added. Finally, magnesium chloride powder (MgCl2) was added to this mixture. The observation is done after 1 minute and a positive result is shown by an effervescence and a brick-red coloration.

Tannins test (Ferric chloride test)

This test was performed according to the method of Banso and Adeyemo (2006). An aliquot of 0.5 g of each lichen powder was mixed with 10 mL of distilled water and filtered. To the filtrate, two drops of 5% iron (III) chloride reagent (FeCl3) were added. The blue-black or blue-green coloration or the formation of a precipitate indicates the presence of tannins.

Alkaloids test (Wagner's test)

This test was performed according to the method of Tiencheu *et al.* (2021). An aliquot of 0.5 g of lichen powders was mixed with 5mL of 1% HCl. After incubation in a water bath for 5 minutes, the mixture was filtered. Two grams (2 g) of potassium iodide and 1.27 g of iodine were dissolved in 5 mL of distilled water and the solution was diluted to 100 mL with distilled water. Two drops of this iodine solution were added to the filtrate; a brown colored precipitate indicates the presence of alkaloids.

Terpenoids test (Salkowski test)

The method described by Ayoola *et al.* (2008) was used to search for terpenoids. To 0.5 g of each lichen grind, 2 mL of chloroform was added. Three milliliter of concentrated sulfuric acid was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

2.3. Lichen extraction

Crude extracts of lichens were prepared as described by Stojanović *et al.* (2010). Approximately 60 g of each lichen powder was subjected to maceration extraction in 600 mL of methanol (80%) for 48 h at room temperature and then the extracts were filtered and concentrated through a rotary evaporator at 40 °C. The obtained dry extracts were stored at 4 °C until use.

2.3.1. Phenolic compounds content

Total phenolic compounds were determined by the Folin-Ciocalteu method (Turkmen *et al.*, 2006). The obtained results were determined from the regression equation of the gallic acid calibration curve (ranging from 0.005 to 0.05 mg/mL) prepared previously and expressed as mg gallic acid equivalents (GAE) per hundred grams of the lichen powder. In this method, 1 mL of methanolic extract of lichens diluted 10-75 times with methanol was mixed with 1 mL of 3-fold diluted Folin-Ciocalteu reagent. Two milliliters of a 35% sodium carbonate solution

were added to the mixture, shaken thoroughly and diluted to 6 mL by adding 2 mL of distilled water. The mixture was allowed to stand for 30 minutes and the formed blue color was measured at 700 nm using a spectrophotometer (Analytik Jena, Specord 50 plus).

2.3.2. Flavonoid content

The flavonoid content of the extracts was determined by spectrophotometry (Djeridane *et al.*, 2006), using the method based on the formation of a flavonoid-aluminum complex, having the maximum absorbance at 430 nm. An aliquot of 1.5 mL of methanolic extract of lichens was mixed with 1.5 mL of a 2% methanolic solution of aluminum chloride. After incubation at room temperature for 15 minutes, the absorbance of the reaction mixture was measured and the flavonoid content is expressed as mg quercetin equivalent (QE) per 100 grams of the lichen powder.

2.3.3. Antioxidant activity

Evaluation of total antioxidant capacity (TAC)

The total antioxidant capacity of the methanolic extracts was assessed by the phospho–molybdate method (Prieto *et al.*, 1999). A 0.5 mL aliquot of diluted lichen methanolic extract was placed in a tube with 5 mL of molybdate reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 minutes. After cooling to room temperature, absorbance was measured at 695 nm against a blank. Antioxidant capacities were expressed as mg ascorbic acid equivalent (AAE) per 100 grams of the lichen powder.

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the methanolic extract was determined according to the method of Gülçin *et al.* (2002). One milliter of diluted lichen methanolic extract was mixed with phosphate buffer (2.5 mL; 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL a 1%). The mixture was incubated at 50°C for 20 min. A 2.5 mL aliquot of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The top layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. The reducing power is expressed as mg ascorbic acid equivalent (AAE) per 100 grams of the lichen powder.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Antioxidant activity was measured as free radical scavenging potential in a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Tepe *et al.* (2005). Fifty microliters of lichen methanolic extract was added to 5 mL of 0.004% DPPH methanolic solution. After an incubation period of 30 minutes at room temperature, the absorbance was read against a blank at 517 nm. The free radical inhibition by DPPH in percent (I %) was calculated as follows:

$$\mathbf{I\ \% = [(A\ blank - A\ sample) / A\ blank] \times 100}$$

A blank: is the absorbance of the control reaction (containing all reagents except the test compound),

A sample: is the absorbance of the test compound.

The results were determined from the regression equation of the calibration curve and are expressed as mg ascorbic acid equivalent (AAE) per 100 grams of the lichen powder.

2.3.4. Antibacterial Activity of methanolic extracts in solid medium

The antibacterial activities of the methanolic extracts were tested against three bacterial strains (methicillin resistant *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) by the solid media diffusion method. After swabbing the bacterial strain (load of 10^8 colony forming units (CFU)/mL) onto Mueller-Hinton agar, Whatman No. 1 paper discs (6 mm diameter) were placed on the surface. Then, 10 µL of each methanolic extract and penicillin as a control (30 mg/mL) were placed on each disk. After incubation at 37 °C for 24 h, the diameters of the inhibition zones around the

discs were measured. The tests were performed in duplicate and the result is expressed as the mean of the standard deviation of the results of both tests (Bekka-Hadji *et al.,* 2022).

2.3.5. Antibacterial Activity of methanolic extracts in a liquid medium

The minimum inhibitory concentrations (MIC) of the methanolic extracts were determined by the microdilution method (Bekka-Hadji *et al.,* 2022). The different concentrations of methanolic extracts and penicillin (0.015 - 15 mg/mL) were carried out in a 96-well round-bottomed microplate. All the tests were carried out with Mueller-Hinton broth at the rate of 100 µL per well. Finally, each well was inoculated with 100 µL of a bacterial suspension (the final load in each well is 10^7 CFU/mL). After 24 h of incubation at 37°C, the MIC corresponded to the minimum concentration showing no bacterial growth.

2.4. Statistical analysis

All data were performed using ORIGIN PRO version 93 E software for Windows. All experiments were performed in duplicate and data obtained from the analysis are expressed as mean ± standard (SD). Statistical differences were analyzed by one-way analysis of variance (ANOVA) at $p < 0.05$ and Fisher LSD test.

3. Results and discussions

3.1. Phytochemical screening

The phytochemical screening, allows to highlight the presence of secondary metabolites at the level of the studied lichen species. The detection of these chemicalcompounds is mainly based on precipitation reactions and specific color change.

The phytochemical evaluation of the different grinds of *Evernia prunastri* species revealed the presence of some chemical constituents such as: alkaloids, flavonoids, saponins, terpenoids, but we note also the absence of tannins (Table 1).

Results reported by Rashmi and Rajkumar (2014), on several lichen species, confirm the presence of at least one known constituent (saponins, alkaloids, terpenoids, flavonoids, etc.) in methanolic extracts. According to these authors, phytochemical analysis performed on lichen extracts revealed the presence of constituents known for their medicinal and physiological activities. The usefulness of lichens is due to the range of secondary compounds they produce.

Table 1. Phytochemical screening of the lichen grind of *Evernia prunastri* samples

	Interferences		
	JS	**SS**	**MS**
Test for saponins (Frothing test)	+	+	+
Test for flavonoids (Cyanidine test)	+	+	+
Test for tannins (Ferric chloride test)	-	-	-
Test for alkaloids (Wagner's test)	+	+	+
Test for terpenoids (Salkowski test)	+	+	+

+ : presence, - : absence, JS: Jijel sample, SS:Setif sample, MS: M'Sila sample.

3.2. Phenolic and flavonoid contents

The contents of phenolic compounds and flavonoids in the methanolic extracts of lichens were determined. The obtained results are presented in Figure 2 (a, b).

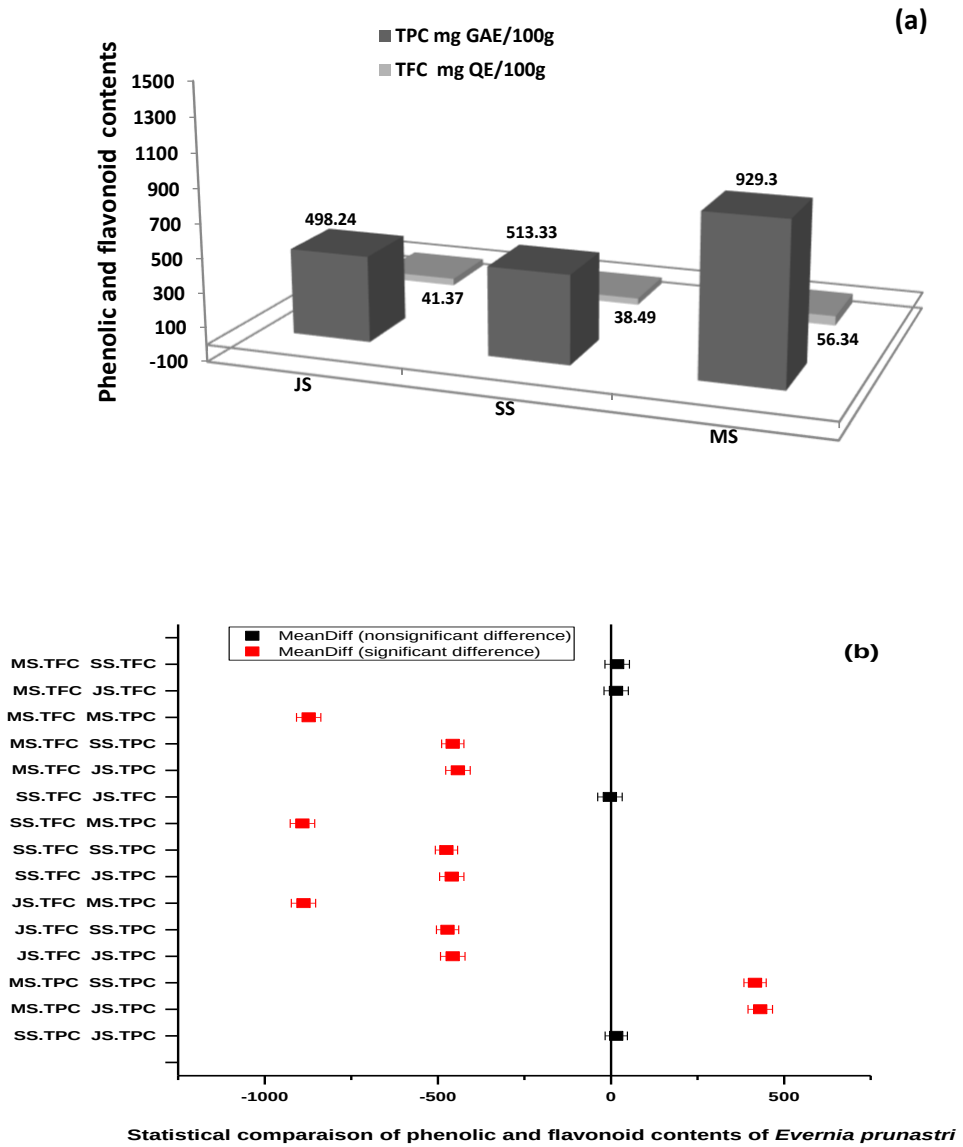

Figure 2. Phenolic and flavonoid contents of methanolic extracts of *Evernia prunastri*s amples **(a)**. Statistical comparison of phenolic and flavonoid contents of *Evernia prunastri* **(b)**. JS: Jijel sample, SS: Setif sample; MS: M'Sila sample; TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalents; QE: Quercetin acid equivalents. Results are expressed as mean ± SD of two experiments for TPC and TFC tests.

The content of phenolic compounds is significantly higher for the lichen from M'Sila (929.3 mg GAE/100g) in comparison with those from Jijel and Setif (498.24 and 513.33 mg GAE/100g, respectively). Mitrović *et al.* (2011), found a phenolic compound content of 80.73 mg/g of the extract of *Evernia prunastri* species from Serbia. This is higher than our phenolic compound result. Similarly, Shcherbakova *et al.* (2021) obtained the highest content (73 mg gallic acid/g extract) for hexane extract of *Evernia prunastri* species collected in Mari El Republic of the Russian Federation. However, Stojanović *et al.* (2010) found a

content of 18.24 µg/g extract for *Evernia prunastri* species from Bojaninevode (Serbia), which is less significant than our result. In comparison with another species of lichen *Xanthoria parietina* collected in Algeria (Boumerdes region), the content of phenolic compounds was found to be 13.9 mg/100g (Bouchenak *et al.*, 2020), which is also lower than our obtained phenolic compound result.

The results of flavonoid contents are presented in Figure 2 (a), the lichen extract from M'Sila region exhibited also the highest content (56.34 mg QE/100g) compared to the other two studied lichen extracts. The flavonoid contents of our extracts are lower than that of lichen *Evernia prunastri* from Serbia (27.46 mg/g) (Mitrović *et al.*, 2011).

3.3. Antioxidant activities of *Evernia prunastri* methanol extracts

The antioxidant capacity (TAC), reducing power (FRAP) and free radical scavenging activity (DPPH) of the lichen methanolic extracts from Jijel, Setif and M'Sila samples were determined. The results obtained are presented in Figures 3, 4 and 5.

3.3.1. Total antioxidant capacity

From the results presented in Figure 3 (a, b), we note that the antioxidant capacity of the methanolic lichen extract of M'Sila (1063.35 mg AAE/100g) is significantly higher compared to those of Jijel and Setif.

The total antioxidant capacities of our extracts are higher than that found by Stojanović *et al.* (2010) for *Evernia prunastri* species (Bojanine vode, Serbia) and which is in the order of 0.60 µg/g. This antioxidant power observed in the extracts may be mainly due to the richness of the extracts in phenolic compounds and it also depends on the chemical nature of the bioactive molecules.

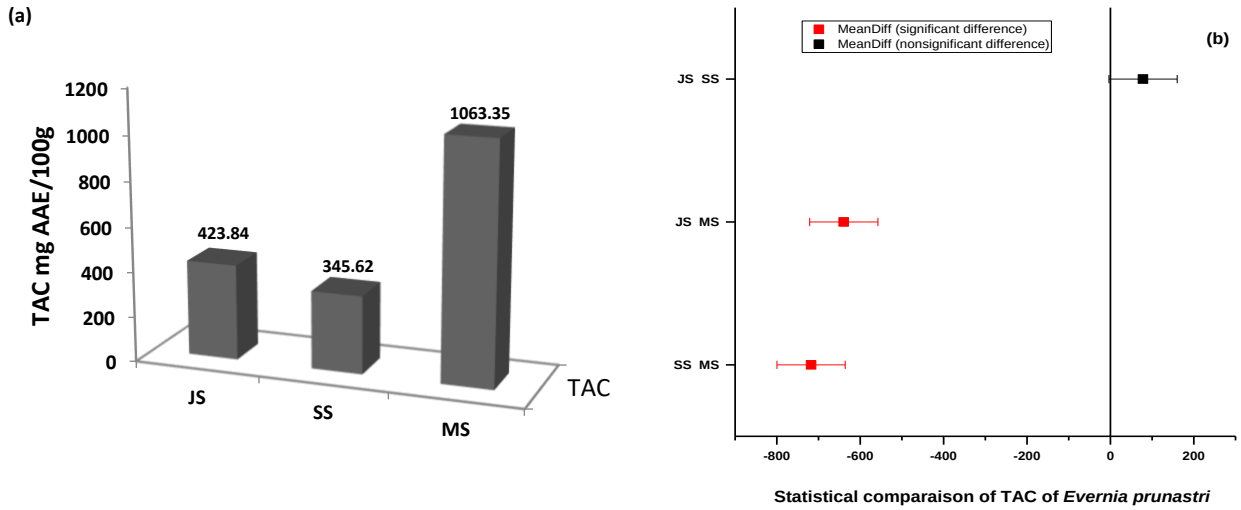

Figure 3. Total Antioxidant Capacity of methanolic extracts of *Evernia prunastri* samples **(a)**. Statistical comparison of TAC contents of *Evernia prunastri* **(b)**. JS: Jijel sample; SS: Setif sample; MS: M'Sila sample; TAC: Total antioxidant capacity; AAE: Ascorbicacid equivalents. Results are expressed as mean ± SD of two experiments for TAC and tests.

3.3.2. Reducing power

The results illustrated in Figure 4 (a, b), revealed that the reducing power of M'Sila lichen sample (277.66 mg AAE/100g powder) is the most important one.

The reducing power of Iron from our extracts is higher than that found by Stojanović *et al.* (2010) for *Evernia prunastri* species from Serbia (35.5 µg/g).

The reducing power of the methanolic

extract of M'Sila lichen sample is slightly higher than that found by Bouchenak *et al.* (2020) who worked on the methanolic extract of *Xanthoria parietina* species (206 mg AAE/100g) from Boumerdes region (Algeria).

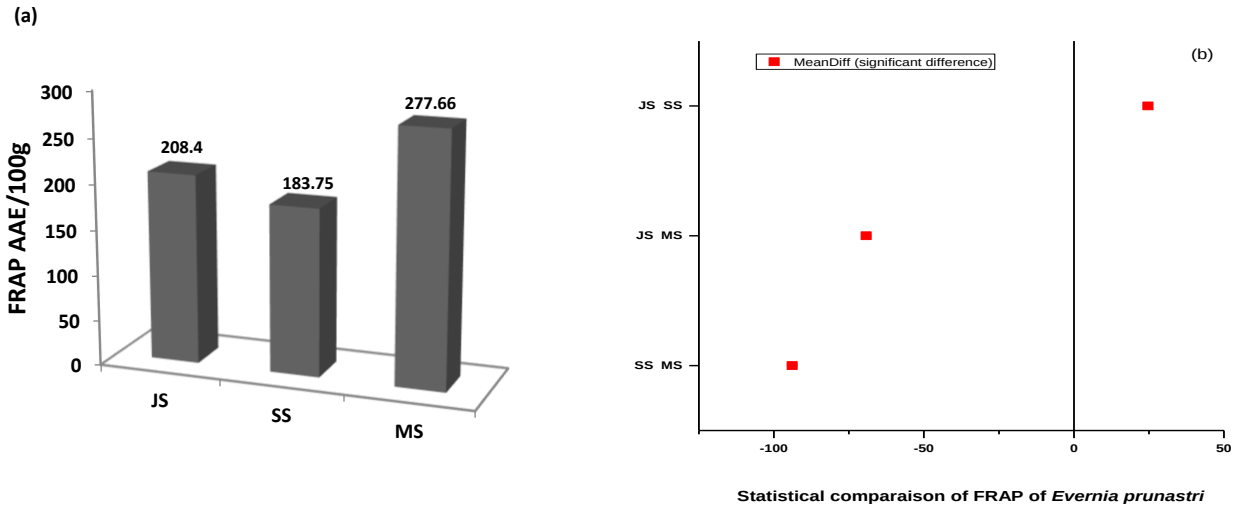

Figure 4. Reducing power of the methanolic extracts of *Evernia prunastri* samples **(a)**. Statistical comparison of FRAP of *Evernia prunastri* **(b)**. JS: Jijel sample; SS: Setif sample; MS: M'Sila sample; FRAP: Ferric reducing antioxidant power; AAE: Ascorbic acid equivalents.Results are expressed as mean ± SD of two experiments for FRAP test.

3.3.3. DPPH scavenging activity

According to our results (Figure 5), the DPPH inhibition rates recorded in the presence of the various methanolic lichen extracts are very weak compared to the antioxidant capacity and the reducing power.

The methanolic extract of the *Evernia prunastri* species growing in the M'Sila region yielded the highest level (7.67 mg AAE/100 g powder). This extract has a high concentration of total phenolic and flavonoids compounds, which correlates with antioxidant activity.

According to Kosanic *et al.* (2013), the high antioxidant activity of the tested lichen extracts correlated with a high content of total phenols, suggesting that phenols are the main agents of their antioxidant activity.

Phenols are very important constituents because of their scavenging capacity due to their hydroxyl groups. These compounds may contribute directly to the antioxidant action. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans (Gülçin *et al.,* 2002). Similarly, flavonoids are a class of secondary metabolites possessing important antioxidant and chelating properties. The antioxidant activity of flavonoids depends on the structure and substitution of hydroxyl groups (Stanković, 2011).

The region of M'Sila constitutes the capital of the Hodna region. The territory of Hodna covers an area of 6951 Km^2, in the heart of one of the largest sets of semi-arid and steppe areas that exist in North Africa. This region is set back from the southern shores of the Mediterranean between the Tellian Atlas in the north and the Saharan Atlas in the south (Mili *et al.,* 2019). This leads us to explain that the production of bioactive substances endowed with antioxidant activity have tendency to be synthesized in conditions of drought and intense light so that the organism can protect itself against adverse conditions.

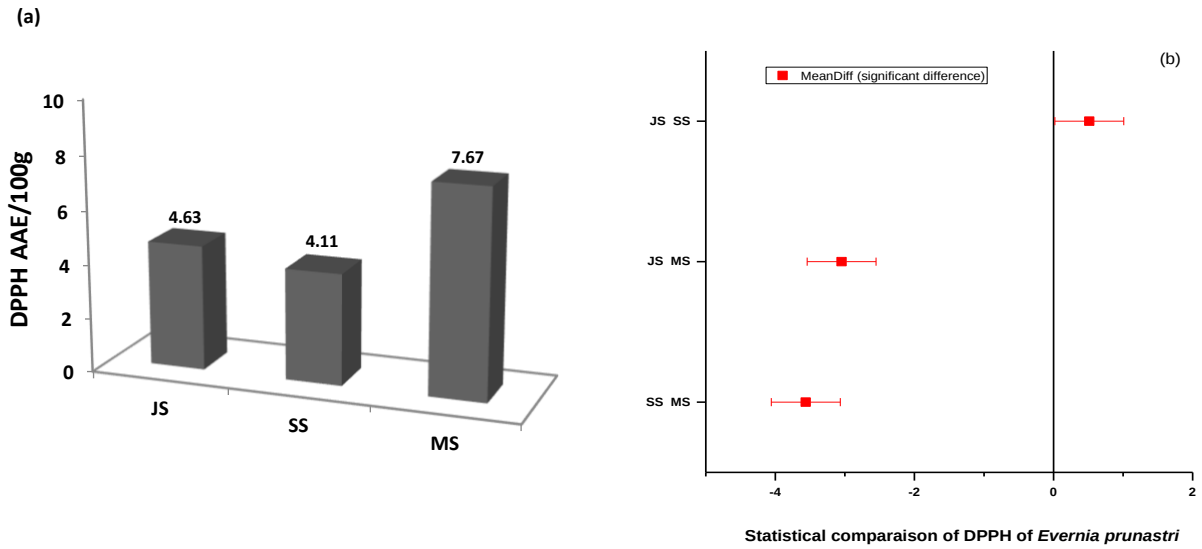

Figure 5. DPPH scavenging activity of the methanol extracts of *Evernia prunastri* samples **(a)**. Statistical comparison of DPPH of *Evernia prunastri* **(b)**. JS: Jijel sample; SS: Setif sample; MS: M'Sila sample; DPPH: 2,2-diphenyl-1-picrylhydrazyl; AAE: Ascorbic acid equivalents. Results are expressed as mean ± SD of two experiments for DPPH test.

3.4. Antibacterial Activities

The antibacterial activities of methanolic extracts of *Evernia prunastri* against three bacteria (methicillin resistant *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) were determined. The obtained results were expressed by the diameters of the inhibition zones (IZDs) and the minimum inhibitory concentrations (MICs). The results are presented in Table 2.

The results show that the methanolic extracts of lichen have a very high antibacterial activity against one of the tested bacterial strain (methicillin resistant *Staphylococcus aureus).* The diameters of the obtained zones of inhibition and the minimum inhibitory concentrations values range from 6 to 35.33 mm and from 0.058 to 15 mg/mL, respectively.

3.4.1. Antibacterial activity of methanolic extracts in solid medium

According to the obtained results, the best activity was obtained for the methicillin-resistant *S. aureus* (MRSA) strain with a diameter of 35.33 mm for the methanolic extract of Jijel lichen. Penicillin used as a control gave a diameter of 35 mm (Table 2, Figure 6).

These results are similar to those of Kiran *et al.* (2013) who found that acetone and chloroform extracts obtained from different samples of the lichen *Evernia prunastri* species from Turkey show antibacterial activity against several strains (*Staphylococcus*, *Enterococcus* and *Bacillus*) with zones of inhibition ranging from 5 to 21 mm for chloroform extracts and from 6 to 20 mm for acetone extracts. For *Staphylococcus* strains, the reported inhibition zones varied from 9 to 20 mm. Likewise, Brakni and Ali Ahmed (2018) investigated the sensitivity of bacteria to different extracts of lichen (*Evernia prunastri* (L.) Ach., *Ramalina fastigiata* (Pers.) Ach. and *Cladonia rangiformis* Hoffm.) collected in the region of Seraïdi (North-East of Algeria). The obtained results showed that the *Staphylococcus aureus* strain was very sensitive to all extracts and more precisely to the methanolic extract of *Evernia prunastri* (an inhibition zone of 43 mm). *Escherichia coli* was sensitive to the chloroformic and acetonic extracts of *E. prunastri* and *R. fastigiata* and to the methanolic and aqueous extracts of *C.*

rangiformis. *Klebsiella pneumonia* was insensitive to all the extracts of lichen, *Proteus mirabilis* and *Pseudomonas aeruginosa* are slightly sensitive to some extracts of *R. fastigiata.*

Table 2. IZDs (mm) and MICs (mg/mL) of the methanolic extracts of *Evernia prunastri* samples and Penicillin control against bacterial strains.

IZDs and MICs of samples lichens and control		**Methicillin resistant *S. aureus*ATCC43300**	***E. coli* ATCC25922**	***P. aeruginosa* ATCC27853**
JS	I Z D MIC	35.33 ± 1.15 0.058	7 ± 0 7.5	8.5 ± 0.7 7.5
SS	I Z D MIC	27.33 ± 1.15 0.12	NZ 7.5	7 ± 0 7.5
MS	I Z D MIC	23.67 ± 1.53 0.12	8 ± 0 7.5	7 ± 0 7.5
P (control)	I Z D MIC	35 ± 1 1.88	20.50 ± 0.35 0.47	NZ >15

JS: Jijel sample; SS: Setif sample; MS: M'Sila sample; P: penicillin; IZD: Inhibition zone diameter (mm) including disk diameter of 6 mm and values are given as mean ± standard deviation; NZ: No inhibition zone; MIC: minimal inhibitory concentration in mg/mL.

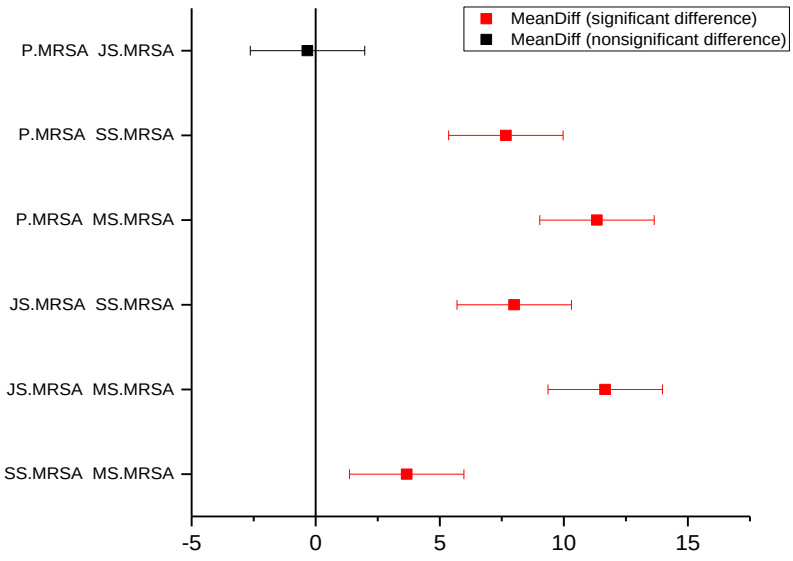

Figure 6. Statistical comparaison of IZDs of the methanolic extracts of *Evernia prunastri* samples and penicillin control against MRSA strain. MRSA: Methicillin resistant *S. aureus* ATCC 43300

3.4.2. Antibacterial activity of methanolic extracts in liquid medium

The MICs of methanolic extracts and penicillin are those obtained in microplate wells before the first growth, i.e. when no growth is visible to the naked eye.

The lowest minimum inhibitory concentrations are those obtained with the methanolic extract of Jijel against *S. aureus* which is of 0.058 mg/mL. However, the highest

MIC was obtained against *E. coli* and *P. aeruginosa* species which is of 7.5 mg/mL and this was observed for all the lichen extracts. In comparison with penicillin, the lowest MIC is retained for *E. coli* with a value of 0.47 mg/mL (Table 2).

Kosanic *et al.* (2013) found that acetone-based extracts of *E. prunastri* from Serbia inhibited all tested microorganisms, but at somewhat higher concentrations. The obtained MICs for the extract of this lichen vary from 6.25 to 25 mg/mL, while for the isolated compound (usnic acid) from this lichen extract, the MICs vary from 0.25 to 1 mg/mL.

The obtained MIC for the *S. aureus* strain is 12.5 mg/mL, while usnic acid gives an MIC of 0.5 mg/mL. This shows that our results are more interesting because the MIC of our extracts is reduced by almost 2 times compared to that of usnic acid. In another study done by Mitrović *et al.* (2011) on methanolic extracts of *E. prunastri* from Serbia, the authors obtained MICs ranging from 0.0391 to 10 mg/mL against all tested strains. The highest MICs (2.5 to 10 mg/mL) were observed for Gram-negative bacteria and the lowest (0.0391 to 0.156 mg/mL) for Gram-positive bacteria. For *S. aureus* strains, the MIC obtained was 0.156 mg/mL. which is close to our MIC result value.

The interesting effect of the tested extracts is probably due to their richness in usnic acid and other active compounds.

The secondary metabolites of lichens are of biological interest for humans as pharmaceuticals. The biosynthesis and the pathways involved in their regulation have been reviewed by a number of authors and many studies have focused on their biological role as protectors of thalli against various stresses and enabling them to withstand unfavorable environmental conditions for their growth (Aoussar *et al.,* 2020).

Shcherbakova *et al.* (2021) showed that the dichloromethane and hexane extracts were both active against *S. aureus* (MICs of 4 and 21 μg/mL, respectively) but they were less active against Gram-negative bacteria and yeasts. The acetone extract showed activity against both *S. aureus* (MIC of 14 μg/mL) and *C. albicans* (MIC of 38 μg/mL).

Several studies have reported the antimicrobial activity of usnic acid, a constituent isolated from lichens, against Gram-positive bacteria including vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, usnic acid is reported to be more effective than penicillin ointments in the treatment of external wounds and burns. Similarly, synergistic action was observed in combination with gentamicin, while antagonism was observed with levofloxacin. The combination with erythromycin showed an indifferent effect (Behera *et al.,* 2005; Araújo *et al.,* 2015).

4. Conclusion

In conclusion, we can say that the tested lichen extracts presented potent antioxidant activities and antibacterial activities especially against *S. aureus*. The substances contained in the extracts included alkaloids, saponins, flavonoids, etc.

This is the first study reporting the *in vitro* biological activities of species of *E. prunastri* collected in three different regions of Algeria. The obtained results of the present study indicate that methanolic extracts obtained from these species are potential sources of natural antibacterial and antioxidant biomolecules. This could be important for their use as antibacterial agents against *Staphylcoccus* infections or as food preservatives against oxidation reactions.

5. References

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