Antibacterial Activity of the Chloroform, Acetone, Methanol and queous Extracts of Algerian Lichens

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ABSTRACT

In the current study, we investigated the antibacterial activity of chloroform, acetone, methanol and aqueous extracts of the following lichens: *Evernia prunastri* (L.) Ach., *Ramalina fastigiata* (Pers.) Ach. and *Cladonia rangiformis* Hoffm. The phytochemical analyses of extracts of each species were determined by thin layer chromatography (TLC) and microcrystallization. These extracts were tested *in vitro* against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Proteus mirabilis and Pseudomonas aeruginosa* by the disc diffusion method.

The sensitivity of the bacteria to the different lichen extracts is as follows: *Staphylococcus aureus* is highly sensitive to all extracts and more precisely to the methanolic extract of *Evernia prunastri* (with a 43 mm inhibition zone), *Escherichia coli* particularly 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* is insensitive to all lichen extracts, *Proteus mirabilis* and *Pseudomonas aeruginosa* are slightly sensitive to certain extracts of *R fastigiata*.

The activity of the lichenic extracts was certainly due to predominant compounds such as lichenic acids (Usnic acid, Evernic acid, Fumarprotocetraric acid and Atranorin), some of which have already proved their antibacterial efficacy. Therefore, these lichens may be used to discover bioactive products that may serve as new sources of natural antimicrobial agents.

Keywords: Lichens, Extract, Secondary metabolites, TLC, Microcrystallization, Antimicrobial activity.

1. INTRODUCTION

Lichens are associations between fungi (mycobionts) and photoautotrophic, algal partners (photobionts). The mycobionte is unique in the symbiotic association and usually dominates it[1]. Lichen symbiosis represents a valuable source for commercially interesting compounds including antimicrobial agents, dyeing agents, spices ingredients and perfume ingredients. Lichens as a group may also produce an amazing array of metabolites, many of which are unique to lichen symbiosis. Generally, lichen metabolites may be divided into two groups: primary and secondary. Primary metabolites are proteins, lipids, carbohydrates and other organic compounds involved in the lichen's metabolism and structure. Secondary metabolites, also known as lichen substances, are mostly small but complex molecules. Many of the traditional medicinal uses of lichens are probably related to their secondary metabolites, many of which are known to both be physiologically active and act as antibiotics[2]. Structures for more than 1,050 different lichen substances have been reported to date[3]. Lichen chemistry continues to be a flourishing branch of natural product chemistry[4]. The

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antimicrobial properties of lichen extracts and their secondary metabolites are known for long[5-11]. Today, lichens are used for many different medicinal purposes, but there are general categories of use that reoccur throughout the world. Lichens are often used externally for dressing wounds, either as disinfectants or to stop bleeding. Other common topical uses are for the treatment of skin infections and the treatment of sores, including sores of the mouth. The importance of its use for topical conditions is apparent in the name "lichen" (from "leikhēn", what eats around itself), which comes from the ancient Greek practice of using cryptogams to cure skin disease[12]. The lichen extracts were tested against common microorganisms were chosen as they are known to cause serious infections and humans diseases: Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*).

The aim of this study was to estimate the antimicrobial activities of acetone, methanol, chloroform and aqueous extracts of three Algerian lichens: *Evernia prunastri, Cladonia rangiformis* and *Ramalina fastigiata* against five bacterial strains. A chemical investigation was also carried out using thin layer chromatography (TLC) and microcrystallization test.

MATERIALS AND METHODS

Lichen sampling and identification

Evernia prunastri (L.) Ach., *Ramalina fastigiata* (Pers.) Ach. and *Cladonia rangiformis* Hoffm. were collected from Seraïdi (a town on the Edough Peninsula in northeastern Algeria) in November 2013 and identified by Pr. Ali Ahmed M, professor of the Biology Department at Annaba University. Identification of the investigated lichens required the spot test. This test has been used universally as a rapid, non-specific means for detecting the presence of certain unspecified lichen substances. The test is convenient and simple to perform, even under field conditions. The color changes occurring at the point of reagent application at the lichen thallus are noted as "+" or "-". These color changes take place due to the presence of a particular secondary metabolite in the thallus, a color change called"spotting"[13,14].

The set of reagents used for the detection of these spots were K, C, KC and PD. K consisted of an aqueous solution of potassium hydroxide (10%). C consisted of a freshly prepared aqueous solution of calcium hypochlorite (10%). KC was applied at a particular spot of the thallus, with K being applied first and immediately followed by C. PD was an alcoholic para-phenylenediamine solution (5%), an excellent reagent for the detection of an aldehyde group in a lichen substance.

Several floras have been used as those of Ozenda and Clauzade, 1970[15], Van Haluwyn et al., 2009[16] and A Database for Rapid Identification of Lichens "LIAS light"[17].

Identification of lichen metabolites

Microcrystallization

Microcrystallization is a useful method to distinguish some lichen compounds. It was developed mainly by Asahina and Shibata, Huneck and Yoshimura[18, 19]. This method is used mostly when a small number of taxa of known chemistry are to be separated[20]. The microcrystal test may be performed on a microscope slide with a high degree of accuracy and speed. In this study, a small fragment of the lichen thallus to be investigated was placed on the middle part of a microscopic glass slide, and one or two drops of acetone were dripped on the fragment by means of a dropper. Following the evaporation of the acetone, lichen substances, if present, were extracted as residue on the slide in the form of a ring around the fragment. The thallus fragment was then removed. Two reagents were then utilized for microcrystallization: water/glycerol/ethanol (1:1:1) and glycerol/acetic acid (1:3)[21]. The slide was heated over a micro-flame and set aside for cooling and crystallization and then the identification of the crystal was carried out by comparing with reference information[19].

Thin layer chromatography (TLC)

Lichen substances have been analyzed using basic methodology previously described in detail in the literature[22-25]. A quantity of 1 mL of acetone was added to a fragment of lichen with a surface area of approximately 1 cm^2, then spotted directly onto TLC aluminum plates (MERCK TLC Silica gel 60 F254). Spots were separated on the plates to a height of 7 cm with different solvent systems. we used three systems, A, E and C because they provide the best discrimination of lichen substances, and it is particularly stable and reliable[27]. Solvent system A contained a mixture of toluene/dioxane/acetic acid (180:45:5, v/v/v) [20], solvent system C contained toluene/acetic acid (170:30, v/v) [23] and solvent system E contained cyclohexane/ethyl acetate (75:25, v/v) [26]. The plates were then pre-equilibrated with 60% acetic acid vapor. The control is the mixture of(usnic acid, atranorin and norstictic acid), we just put the 3 in the same spot, it help us to detect this molecule into our extract, because its Rf is known. The plates were examined under short wavelength (254 nm) and long wavelength (350 nm) ultraviolet light. After brief drying, the plates were sprayed with 10% sulfuric acid until wet (but with no run-off), and then heated at 110 C° in an oven or on a hotplate for 10 minutes to develop the spots. The Rf(rate of flow) values and the diagnostic color of each lichen substance were recorded[27]. The formula for Rf was calculated as follows:

$$Rf = \frac{\text{Distance travelled by lichen substance (indicated by spot)}}{\text{Distance travelled by solvent (indicated by solvent front)}}$$

Estimation of lichen antimicrobial activity

Preparation of extracts

The lichen thalli were thoroughly washed, spread on paper sheets and dried in the lab. The dried material was powdered in an electric grinder. To prepare the 3 types of lichen solution, 10-g amounts of this powder were each added to separate, 100-mL amounts of the solvents acetone, methanol and chloroform (10g/100mL, w/v) and left for 2 days at room temperature (20±2°C).To prepare the aqueous extract, 10 g of lichen powder were added to 100 mL of distilled water with a Soxlhet extractor for 7 hours at 80°C[28]. Thus, a total of 12 lichen extracts were prepared.

After that, each extract was passed through filter paper (Whatman No.1), and the final filtrate was transferred to a rotary evaporator (Büchi Rotavapor R-114). Solvent evaporation was carried out at the specific boiling temperature of each solvent (56˚C for acetone, 65˚C for methanol and 61˚C for chloroform) for 48 hours for complete extraction of secondary compounds[24].The aqueous extract was evaporated in a water bath at 80°C. The extracts were then tested for antibacterial activity against pathogenic bacteria.

Antibacterial Assay

The microorganisms that have been selected are (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus*) have been obtained from the infection laboratory (University Hospital Ibn rochd Annaba, Algeria). The antimicrobial test was carried out using Agar well diffusion methods[29]. Test solutions of lichen substances were prepared by dissolving 10mg of lichen substances in 1 mL of their respective solvents (10mg/mL).

Muller-Hinton agar media was poured into Petri plates. A small, sterile cotton swab was dipped into the 24-hour old culture of bacteria. Then, the dried surfaces of the plates were inoculated by streaking the swab over the entire sterile agar surface. After inoculation, the plates were allowed to dry at room temperature (20±2°C) for 15 minutes. Sterile paper disks (6 mm in diameter) were impregnated with 20 µL of the lichen extract. The negative control consisted of 20µL of pure extraction solvent. The plates were incubated at 37°C in dark conditions. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition (in millimeters) around the disk after 24 hours. The experiments were carried out in triplicate[28].

RESULTS AND DISCUSSION

Botanical description of the species studied

The different harvested species of lichen are described in Table 1.

Depending on the species, the yield varied between 10.3% for *Cladonia rangiformis* and 30.9% for *Ramalina fastigiata.* We observed that extraction with water gave a very good yield for *Evernia prunastri* (30.5%) as well as for *Cladonia rangiformis* (30.2%), although the latter gave a poor yield when extracted with methanol (Figure 1).The differences in the extract yields from the tested lichen materials in the present analysis might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of lichens.

Table 1. Taxonomic description of lichen species (photos: specimen of Edough, description: flora cited above)

Evernia prunastri	*Ramalina fastigiata*	*Cladonia rangiformis*
		
o Thallus fruticose, small bush forming, 3 to 6 cm long, lobes rather soft and in general pendulous, branched, with tips palmated. Upper surface green-grey, lower surface minutely cannulated. Photobiont: chlorococcoide. o Farinose soralia. Apothecia very rare, on top of stipe, 2 to 5 mm diameter, disc brownish. o C-, K+ yellow on both faces, KC-, PD-	o Thallus tufted, often half-spherical, 2 to 5 cm tall, more or less erect, and made of short branches. Upper and lower surface concolorous, pale grey-greenish. Photobiont: trebouxioid. o Apothecia numerous, at lobe apices, disc concave, then flat and occasionally convex. o Chemical spot tests negative.	o Primary thallus made of small basal squamules, rare, vanishing soon and sometimes absent, upper surface grey-greenish. Photobiont : trebouxioide. o Podetia forming tufts up to 5 to 7 cm tall, densely branched with acute angles. o C-, K+ yellow, PD+ red, KC-.

Identification of lichen metabolites

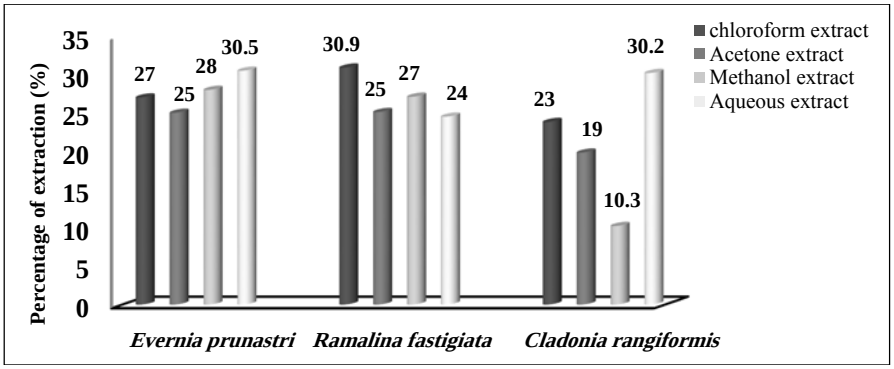


Figure 1: Soxlhet extracts yield (g) of selected lichen species

Major chemical compounds identified from lichen extracts with the microcrystallization test are reported in

Figures 2, 3 and 4.The microcrystallization method relies on the characteristic crystal forms assumed by lichen substances when recrystallized in a suitable solvent[20, 21].

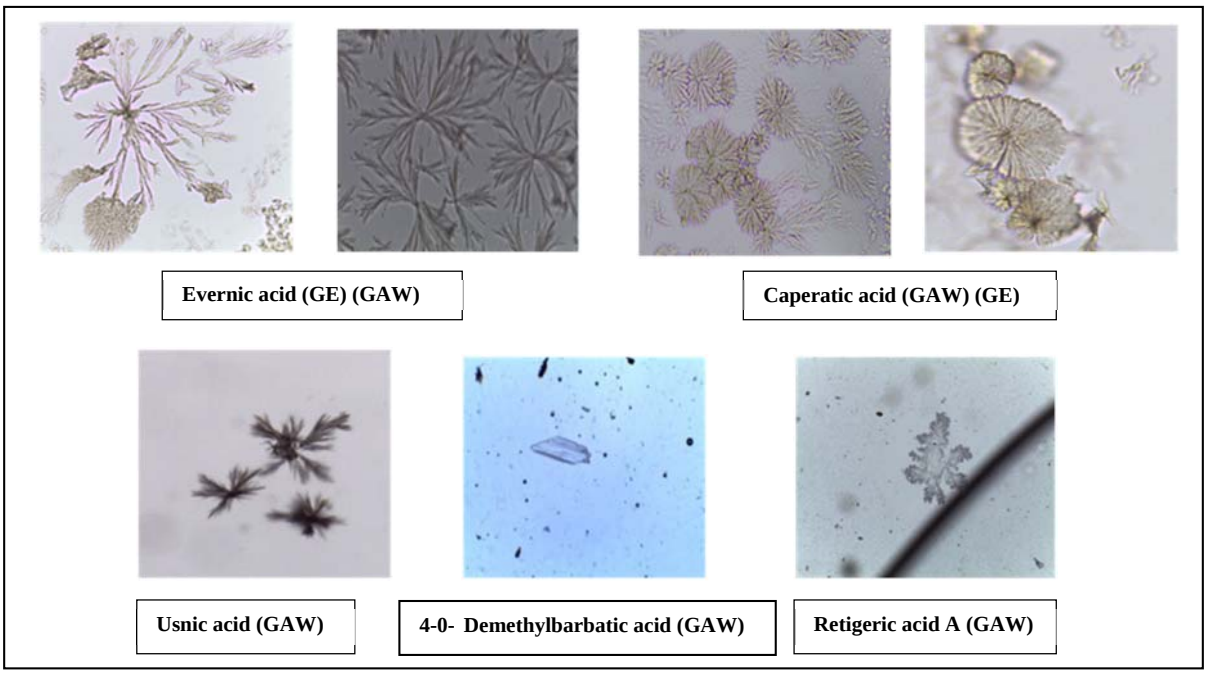


Figure 2: Major chemical compounds from *Evernia prunastri* identified through microcrystallization. GAW: H20: glycerol: ethanol = 1: 1: 1 (v/v/v) / GE: acetic acid: glycerol = 1: 3

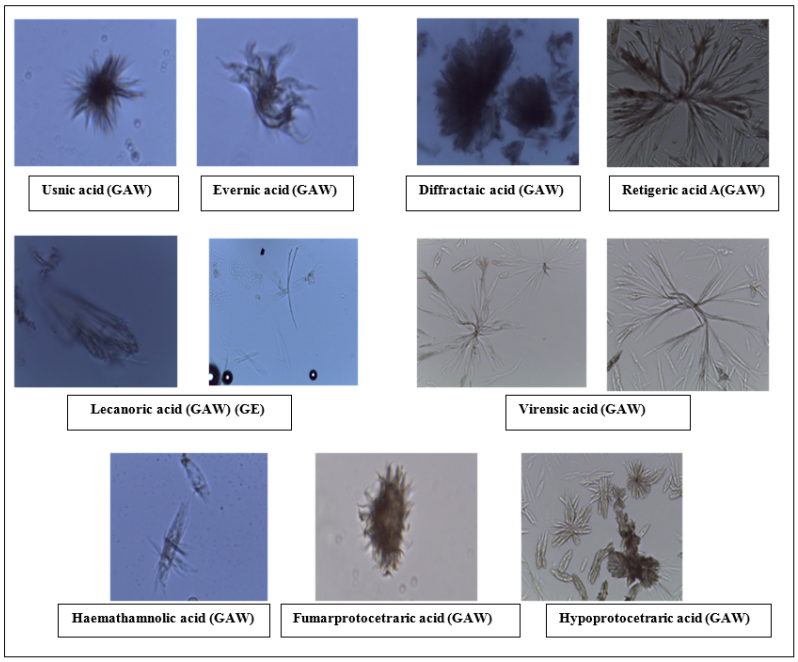
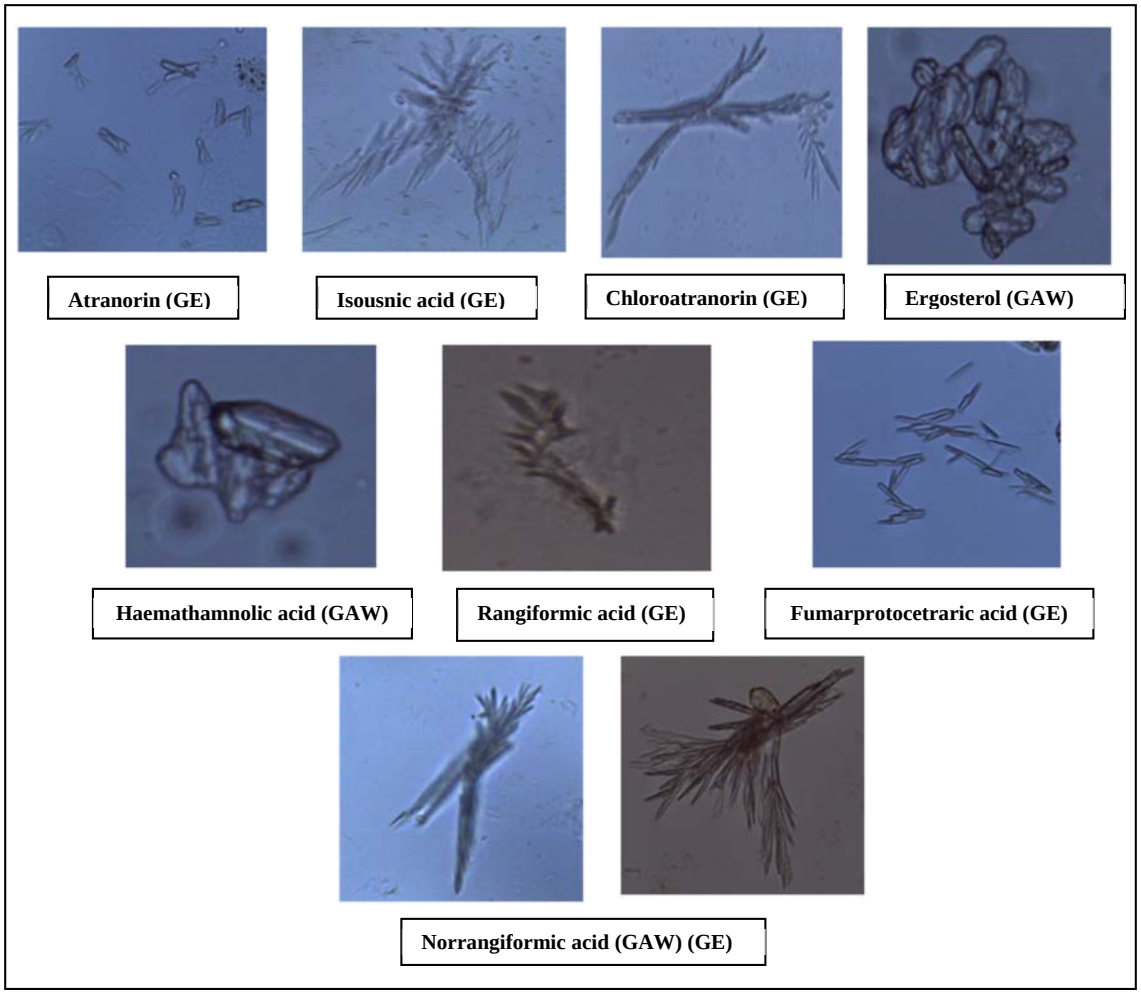


Figure 3. Major chemical compounds from *Ramalina fastigiata* identified through microcrystallization. GAW: H20: glycerol: ethanol = 1: 1: 1 (v/v/v) / GE: acetic acid: glycerol = 1: 3



GAW: H20: glycerol: ethanol = 1: 1: 1 (v/v/v) / GE: acetic acid: glycerol = 1: 3

Figure 4: Major chemical compounds from *Cladonia rangiformis* identified through microcrystallization

The different lichenic compounds identified by TLC are presented in the table 2 the identification was made according to the literature [30].

Table 2. Lichen compounds of different species studied identified using thin layer chromatography

Lichen species	Rf			Color	Identified compound
	A	C	E		
Evernia prunastri	-	27	-	Pale Orange	Evernic Acid
	-	51	-	Orange	Sekikaic Acid
	73	71	-	Orange	Atranorin
	98	-	-	Orange	Unknown
	68	-	-	Green	Usnic Acid
	-	-	19	Yellow	Unknown
	-	-	50	Purple	Unknown
	-	-	95	Purple	Unknown
Ramalina fastigiata	-	24	-	Pale Orange	Evernic Acid
	-	32	-	Yellow	Norstictic Acid
	-	43	-	Grey	Unknown
	-	51	-	Pale Orange	Methyl B-Orcinol carboxylate 4o-Methylhypoprotocetraric Acid
	-	44	-	Grey	
	70	63	24	Green	Usnic Acid
	-	94	-	Grey	Unknown
	80	-	-	Pink	Unknown
	74	-	-	Orange	Atranorin
	67	-	-	Orange	Unknown
Cladonia rangiformis	72	71	-	Orange	Atranorin
	-	98	93	Orange	Unknown
	-	80	-	Pink	Unknown
Control (norstictic acid+ atranorin+ usnic acid	74	75	-	Orange	Atranorin
	69	70	24	Green	Usnic Acid

R_f: front report, the value of R_f is not an average
A, C, E: Different systems

We observed that usually lichenic acids that have been identified by microcrystallization (fig 2 and 4) do not appear in the table2 this is due to the fact that the Microcrystallization is a useful method to distinguish some compounds which separate poorly in TLC systems. We were able to identify 7 metabolites in *E prunastri*, 13 in *R fastigiata* and 9 in *C rangiformis* (Table 3).

Table 3. Secondary metabolites identified through microcrystallization and thin layer chromatography

Lichen species	Compounds	Class
Evernia prunastri	Evernic acid	Orcinol Depsides
	Sekikaic acid	Orcinol Depsides
	Usnic acid	Dibenzofurans
	Atranorin	β-Orcinol Depsides
	4-*O*-Demethylbarbatic acid	β-Orcinol Depsides
	Caperatic acid	Aliphatic acids
	Retigeric acid A	Terpenoids
Ramalina fastigiata	Usnic acid	Dibenzofurans
	Evernic acid	Orcinol Depsides
	Lecanoric acid	Orcinol Depsides
	Norstictic acid	β-Orcinol Depsidones
	Fumarprotocetraric acid	β-Orcinol Depsidones
	Hypoprotocetraric acid	β-Orcinol Depsidones
	Virensic acid	β-Orcinol Depsidones
	Methyl β-orcinol-carboxylate	Monocyclic aromatic derivatives
	Atranorin	β-Orcinol Depsides
	4-*O*-Methylhypoprotocetraric	β-Orcinol Depsides
	Diffractaic acid	β-Orcinol Depsides
	Haemathamnolic acid	β-Orcinol Depsides
	Retigeric acid	Terpenoids
Cladonia rangiformis	Atranorin	β-Orcinol Depsides
	Chloroatranorin	β-Orcinol Depsides
	Haemathamnolic acid	β-Orcinol Depsides
	Rangiformic acid	Aliphatic acids
	Norrangiformic acid	Aliphatic acids
	Fumarprotocetraric acid	β-Orcinol Depsidones
	Hypoprotocetraric acid	β-Orcinol Depsidones
	Ergosterol	Steroids
	Isousnic acid	Usnic acid derivatives

The majority of the lichenic metabolites identified by the use of the techniques: microcrystallization and TLC. This later belongs to two famous classes' depside and depsidone (Table 3). We found the presence of usnic acid in *E. prunastri* and *R. fastigiata,* and its isomer (isousnic acid) in *C. rangiformis.* The retigeric acid A (of the terpenoids class) was present only in *E. prunastri* and *R. fastigiata*. The steroids were represented by ergosterol in *C. rangiformis*, while the aliphatic acids were represented by rangiformic acid and norrangiformic acid, also in *C. rangiformis*. We found atranorin in all three lichen species.

Previously published results showed that *E. prunastri* extracts are characterized by the presence of evernic acid, atranorin and usnic acid[31, 32], which were consistent with

the results of this study. *R. fastigiata* is characterized by the presence of fumarprotocetraric acid, lecanoric acid, evernic acid, usnic acid and stictic acid, which were mostly consistent with the findings of this study. The only exception was stictic acid, which was not detected; however, the derivate norstictic acid was detected[33]. The presence of rangiformic acid, fumarprotocetraric acid, norrangiformic acid and atranorin in the lichen *C. rangiformis* has been reported[34], these compounds characteristic of *this specie*[17] and these were consistent with the results of this study. We also identified Isousnic acid, an isomer of usnic acid[35], has previously been isolated from *Cladonia pleurota*[32]. Isousnic acid differs from usnic acid by the inversion of the group at C6 and C9 in the ring A[36]. Usnic acid is widely distributed in species of *Cladonia* (Cladoniaceae) [37, 38].

The hydrolysis of both evernic acid and atranorin produces methyl β-orcinol carboxylate, which was detected by TLC in the *R fastigiata* extract. Haemathamnolic acid present in *C rangiformis* and even in *R fastigiata* is also a hydrolytic product of atranorin[39].

Antibacterial activity analysis

The antibacterial activity of the tested lichen extracts against the tested microorganisms is shown in Table 4.

Table 4. The antibacterial activity of investigated lichen extracts of chloroform (A), acetone (B), methanol(C) and aqueous (D).The average value indicate the diameter of the inhibition zone (mm)

Species	***Evernia prunastri***(L.) Ach.				***Ramalina fastigiata***(Pers.) Ach.				***Cladonia rangiformis***Hoffm.			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Staphylococcus aureus	18±1.41	17±2.64	43.66±2.08	0.0	21.5±2.12	5.5±0.7	14±2.82	30±0.0	20.33±0.57	5±1.52	21±0.81	23±2.08
Klebsiella Pneumonia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proteus Mirabilis	0.0	0.0	0.0	0.0	0.0	6±1.41	0.0	0.0	0.0	0.0	0.0	0.0
Escherichia coli	8.5±1.2	10±2.3	0.0	0.0	9±0.41	15±0.0	0.0	6.5±1.41	0.0	0.0	11±2.82	3±0.50
Pseudomonas aeruginosa	0.0	0.0	0.0	0.0	2±0.00	6±1.41	0.0	0.0	0.0	0.0	7±1.41	0.0

The results obtained in this study indicate differences in antimicrobial activity among the extracts depending on the species of lichen, species of bacteria and type of extracting solvent.

Based on type of extract

Many researchers have found strong antimicrobial activity of acetone and methanolic extracts when compared to aqueous extracts[40-43]. However, in this study, the aqueous extracts showed significant activity. The aqueous extracts of *R fastigiata* as well as *C rangiformis* were active against the bacteria *S. aureus*, but had the least activity against *E. coli*. Only the aqueous extract of *E prunastri* was totally inactive against all the microorganisms tested. The strongest antibacterial activity of the methanol extract compared to other extracts consistent with (Mitrovic et al., 2011, bezivin et al., 2000)[3,44] and this can be explained by the presence of polar compounds which is mostly found in the methanol extract of lichens[40,9]. This could be also explain that *Evernia* compounds are not hydrosolule.

Based on bacteria species

Staphyloccoccus aureus showed the greatest sensitivity to the lichen species tested. Its largest zones of inhibition were measured at 43.66mm for the methanol extract of *E. prunastri* and 30mm for the aqueous extract of *R. fastigiata*. All the extracts of the exploited lichen were active against *Staphyloccoccus aureus* except the aqueous extract of the *Evernia prunastri* did not exhibit an

inhibition zone. The sensibility of the gram (+) bacteria *Staphyloccoccus aureus* against different lichen species has been reported[10,35,45,46].

The acetone extracts showed the best activity against *Escherichia coli* (15mm for *R fastigiata* and 10mm for *E prunastri).* On the other hand, the acetone extract of *C rangiformis* showed no activity. Mean while, the methanol extract of *C rangiformis* showed considerable activity (11mm) against *E. coli*. The acetone extract was more active than the methanol extract in several previous researches[47,48]. The activity of the acetone extract may be due to the presence of two common compound in *R fastigiata* and *E prunastri* (depside evernic acid and dibenzofuran usnic acid) this tow molecules has proved its antimicrobial activities [49,33]. Minimum activity was shown against *Pseudomonas aeruginosa* (7mm for the methanol extract of *C rangiformis* and 6mm for the acetone extract of *R fastigiata*).Trace activity was shown (2mm) for the chloroform extract of *R fastigiata*. Trace activity was noted against *Proteus mirabilis* (6mm for acetone extract of *R fastigiata*). The antimicrobial activity of *R fastigiata* is related to the presence of the depsidone fumarprotocetraric that has been reported in the literature[40,33]. The bacterium *Klebsiella pneumonia* was resistant to all extracts of the explored lichen. Karagöz et al., 2009[50] have also produce a similar results *Klebsiella pneumoniae was resistant against 11 lichen species.* In contrast, *Kosanic et al, 2014[51] have reported the activity of Cladonia sp against Klebsiella pneumonia.*

Based on lichen metabolites

The activity of the various lichenic extracts against the different bacterial strains studied was primarily due to the activity of the lichenic acids contained in these various extracts. Lawrey, 1986[52] had mentioned that usnic acid and evernic acid inhibit the growth of *Staphylococcus aureus*, but they have no effect on *Pseudomonas aeruginosa*. Also, the strong activity of usnic acid, atranorin and fumarprotocetraric contained in *Cladonia foliacea* against *Staphylococcus aureus* and *Proteus vulgaris* have been reported by Yilmaz and colleagues[47].The activity of evernic acid against Gram-positive bacteria was reported by Rezanka et *al*[53] which explains the strong activity of the extract of *Evernia prunastri*. The extracts of *R fastigiata* were the most active among the extracts of other species, likely due to the presence of evernic acid, fumarprotocetraric and lecanoric acid[33].

Conclusion

In conclusion, the chemical tests carried out allowed us to identify a rich composition of lichen metabolites in the extracts. *Evernia prunastri* acetone extract was the most actif lichen extracts. On other hand, aqueous extract from *Ramalina fastigiata* and *Cladonia rangiformis* were showed the highest activity between extracts of the same species. *Klebsiella pneumonia* showed resistance against all the extract of lichen. These three Algerian lichens merit further investigation in order to identify their chemical composition, purified the lichen metabolites and to determine their mode of action against bacterial strains.

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النشاط المضاد للبكتيريا للكلوروفورم، الأسيتون، الميثانول والمستخلصات المائية لبعض الأشنات الموجودة في الجزائر

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ملخص

تهدف الدراسة إلى التحقق من النشاط المضاد للبكتيريا بواسطة الكلوروفورم، الأسيتون، الميثانول ومستخلص المائي للاشنات Evernia prunastri (L.) Ach., Ramalina fastigiata (Pers.), Cladonia rangiformis Hoffm. التحاليل الفيتوكيميائية لمستخلصات كل نوع تم تحديدها بواسطة كروماتوغرافيا الطبقة الرقيقة (TLC) والبلورة الجزئية (microcrystallization).

لقد تم اختبار الفعالية البيولوجية للمستخلصات المتحصل عليها ضد أنواع مختلفة من البكتيريا ألا وهي Klebsiella pnemonia, Proteus mirabilis, Pseudomonas aeroginosa, Staphylococcus aureus و Escherichia coli.

باستعمال طريقة نشر الأقراص على وسط مغذٍّ صلب. على الرغم من أن Klebsiella Pneumonia كانت مقاومة لجميع مستخلصات الأشنات، لكن بالمقابل Staphylococus aureus كانت حساسة جدا وخاصة بالنسبة للمستخلص المائي Ramalina fastigiata (قطر منطقة تثبيط 30 ملم). عموماً، كانت البكتيريا Escherichia coli حساسة لجميع المستخلصات الاشنيه وأخيراً حساسية منخفضة من Proteus mirabilis و Pseudomonas aeruginosa ضد مستخلصات Ramalina fastigiata و Cladonia rangiformis.

إن نشاط المستخلصات الاشنيه يرجع إلى المركبات السائدة مثل الأحماض الاشنيه (usnic acid, evernic acid, atranorine)، وهذا وفقا للدراسات السابقة التي أكدت قدره هذه المركبات كمضادة للبكتيريا. ولذلك فان هذه الاشنات يمكن استخدامها لاستخلاص منتجات ومكونات حيوية نشطة التي قد تستخدم كمصادر جديدة وموارد طبيعية للمضادات الحيوية.

الكلمات الدالة: مستخلصات الأشنات، نواتج الايض الثانوية، كروماتوغرافيا الطبقة الرقيقة، البلورة الجزيئية، النشاط المضاد للبكتيريا.

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