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Original Research Article

Chemical composition and antimicrobial activity of *Evernia prunastri* and *Ramalina farinacea* from Algeria

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The chemical composition of essential oil, isolated from *Evernia prunastri* and *Ramalina farinacea* by hydrodistillation, was analysed by GC and GC/MS. A total 32 compounds representing 93.64% of the oil were identified in *E. prunastri.* This oil is characterised by an n-octadecanol (54.06%), n-tetradecanol (8.27%), Heptadecane (4.70%), Eicosene-1 (3.82%), n-hexadecanol (2.32%), Tridecenol acetate (2.05%). *Ramalina farinacea* with 34 compounds, representing 76.04% of the total oil, is characterised by the Manool (14.21%), n-octadecanol (10.24%), Eicosene-1 (8.26%), n-tetradecanol (7.68%), Manool oxide-13-epi (5.40%), α-pinene (4.04%), Abietal (3.75%). To test the antibacterial activity of essential oil of these species, four bacteria are used in this study. The oils have shown little effect against gram-negative bacteria, and modest antibacterial activity against Gram positive bacteria.

Keywords: Lichens, *Evernia prunastri*, *Ramalina farinacea*, chemical composition, antibacterial activity, Babors, Algeria.

INTRODUCTION

Lichens are the symbiotic association of fungi and a photosynthetic partner, either a green algae or a cynobacterium. The lichen's name refers to their fungal components, about 18000 lichen species are known. They are important constituents of many ecosystems. They usually grow on rocks and non-fertile ground and as epiphytes on trees and leaves (Seymour et al., 2005). Several lichens are identified as aromatic species (Liu et al., 2014). They provide a great variety of metabolic products, some of which appear to occur naturally only in lichens, while others are also present in higher plants and fungi. Their secondary products play a dominant role in the systematic of lichen. These metabolites sometimes account for more than 30% of the dry mass of the thallus. The volatile part of the extracts might represent a minor proportion of the constituents. On the other hand, good software (such as NIST, 2002) for searching various MS libraries (Adams, 2007; MC Lafferty et al., 2004) enables constituent identification by GC–MS for many known compounds without isolation and standards (Stojanovic et al., 2011).

The lichen secondary metabolites are from derived of mycobiont metabolism, are organized into several distinct chemical classes (Johnson et al., 2011; Manojlovic et al., 2012), such as depsides, depsidones, dibenzofurans and phenolic compounds, most of which are not known from other groups of plants. The biological potential of the lichens has been proven through their use in folk medicine. The lichens are used for human and animal nutrition and in the production of colours, perfumes and alcohol Lichens have also been used in many countries as a cure for diseases in humans such as jaundice, pulmonary, stomach and cranial diseases (Goyal et al., 2016).

Lichens and their metabolites have manifold biological activities: antipyretic (Ingolfsdottir, 2002), antioxidant (Ozen and Kinaliogla, 2008; Luo et al., 2010; Kosanic et al., 2012b; Marijana et al., 2013), antitumor (Malhotra et al., 2008), anti-inflammatory (Süleyman et al., 2002),

antimicrobial (Marijiana et al., 2010; Bahar et al., 2012), antiviral antibiotic allergenic, plant growth inhibitory, antiherbivore, ecological and enzyme inhibitory (Huneck, 1999; Karagoz et al., 2009; Kosanic and Rankovic, 2011; Kosanic et al., 2012b), and analgesic (Bugni et al., 2009).

Lichens may be used as possible natural antioxidant, antimicrobial and anticancer agents (Kosanic et al., 2012b, Manojlović et al., 2012; Ritika and Jayanthi, 2013). Lichens tested, showed a relatively high antibacterial activity (Dzomba et al., 2012; Tatjana et al., 2014; Tahereh and Minoo, 2015). The extracts of lichens tested, showed strong activity, antioxidant, antimicrobial and anti-cancer (Marijana et al., 2010, 2013).

The secondary metabolites have important biological activities such as antipyretic (Ingólfsdóttir, 2002), cytotoxic (Bezivin et al., 2003, 2004), antiinflamatuar (Süleyman et al., 2002), antitumor (Malhotra et al., 2008), analgesic (Bugni et al., 2009), and antioxidant (Luo et al., 2010; Rankovic et al., 2011). Malaysian lichens showed a very strong antioxidant activity (Stanly et al., 2011).

Many lichens were used as a remedy for pulmonary tuberculosis and in the treatment of wounds and skin disorders, owing to pronounced antimicrobial activity of some of their secondary metabolites (Halama and Haluwin, 2004). In addition, lichens have also been identified as a source of biologically active enzymes, polysaccharides and fatty acids that may have pharmacological potential (Huneck and Yoshimura, 1996; Johnson et al., 2011). Lichen substances exert a wide variety of biological actions including antibiotic, antimycotic, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects (Manojlovic et al., 2010a, b; Kosanic et al., 2012a; Pavlovic et al., 2012). *Lobaria pulmonaria* and *P. sulcata* have been used in the treatment of pulmonary and cranial diseases respectively, *Xanthoria parietina* has been used to cure jaundice and *Letharia vulpina* has been used in the treatment of stomach diseases (Huneck, 1999; Kirmizigul et al., 2003; Malhotra et al., 2008). The usage of some lichens in traditional medicine for many years has been justified by subsequent research confirming their various biological activities.

The aim of the present work is the extraction and the identification of the compounds of essential oil of two species *Evernia prunastri* and *Ramalina farinacea* by CG/MS, and tested the antibacterial activity of secondary metabolites of these lichens.

MATERIALS AND METHODS

Plant material

Lichen samples of *Evernia prunastri* (L.) Ach. and *Ramalina farinacea* (L.) Ach. (Figure 1 and 2) were collected from Babors Mountains (Setif, Algeria) (Figure 3) in September 2014. The voucher specimens were deposited in the herbarium of the Department of Biology and Ecology Vegetal, Setif-1 University, Algeria. The air dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type. The oil obtained was collected and dried over anhydrous sodium sulphate and stored at 4°C in sealed brown vials until use.

Essential oil analysis

The essential oils were analysed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25 µm), programming from 50°C (5 min) to 300°C at 5°C/min. with a 5 min hold. Helium was used as the carrier gas (1.0 ml/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280°C, respectively. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the m/z range 33450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library (Masada, 1976; NIST, 2002; MC Lafferty et al., 2004), and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values (Adams, 2007).

Antibacterial activity

Two gram negative and two gram positive bacteria strains were used to determine the antibacterial activity of essential oil of *E. prunastri and R. farinacea*. Bacteria are used in the study as follows: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* 25922 and *Pseudomonas aeruginosa* ATCC 27853. The anti-bacterial activity of the essential oils was determined by the disc diffusion method (Cockerill et al., 2012). The inoculums contain 2.0 × 10 CFU/mL of bacteria. Sterile absorbing paper discs (6 mm in diameter) were impregnated with 10 µl of different oil dilutions (1, 1/2 and 1/5 v/v) in Dimethyl sulfoxide (DMSO), and then placed on the surface of inoculated Petri dishes (90 mm). The diameter of inhibition was measured after 24 h of incubation at 37°C. Gentamicin [10µg/mL and DMSO were used as positive and negative controls, respectively. The diameters of the inhibition zones were measured in mm. All the growth inhibition tests were performed in triplicate.

RESULTS

The essential oil, of two lichens species, isolated by hydrodistillation from the aerial parts, was obtained in very low yield 0.01% (v/w). The analysis by gas chromatography/mass spectrometry (GC-MS) (Figure 4 and 5) of the chemical composition of essential oils, we allowed the identification of 32 compounds in oil of *E. prunastri*, representing 93.64% of the total oil, and 34 compounds representing 76.04% of the total oil of *R. farinacea.*

The compounds, identified in these oils and their relative

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

Figure 2: *Ramalina farinacea* (L.) Ach.

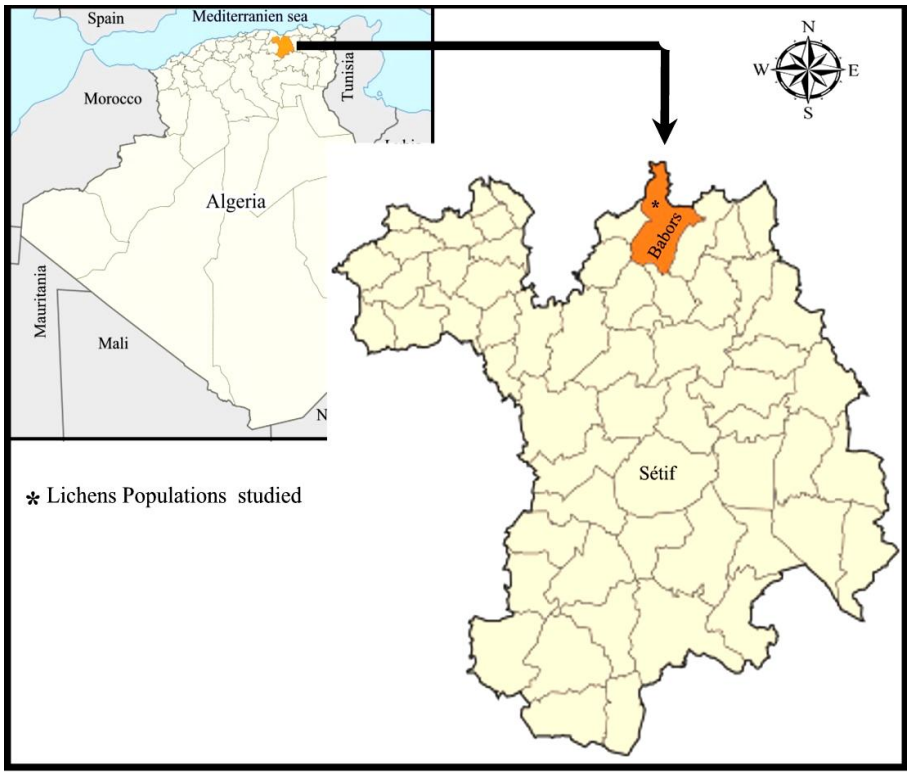

Figure 3: Populations of lichens studied

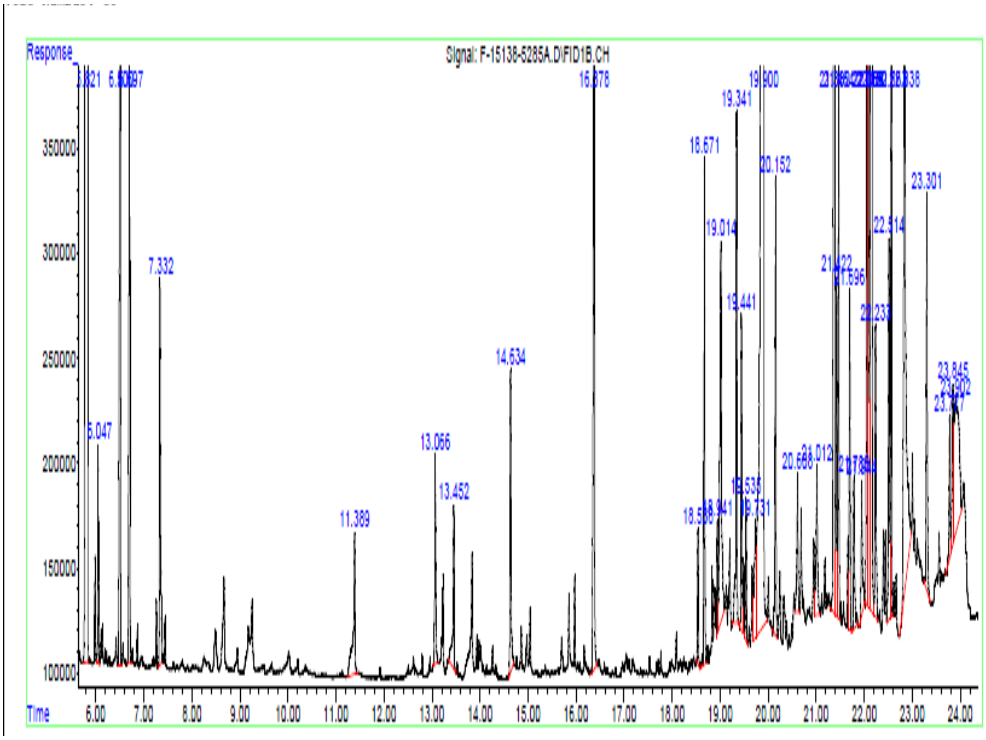

Figure 4: GC/FID profiles of *E. prunastri*

abundance, are presented in their order of appearance (Table 1). Both species studied, *E. prunastri* and *R. farinacea*, are characterized by the presence of common major components, n-octadecanol (54.06%-10.24%), n-

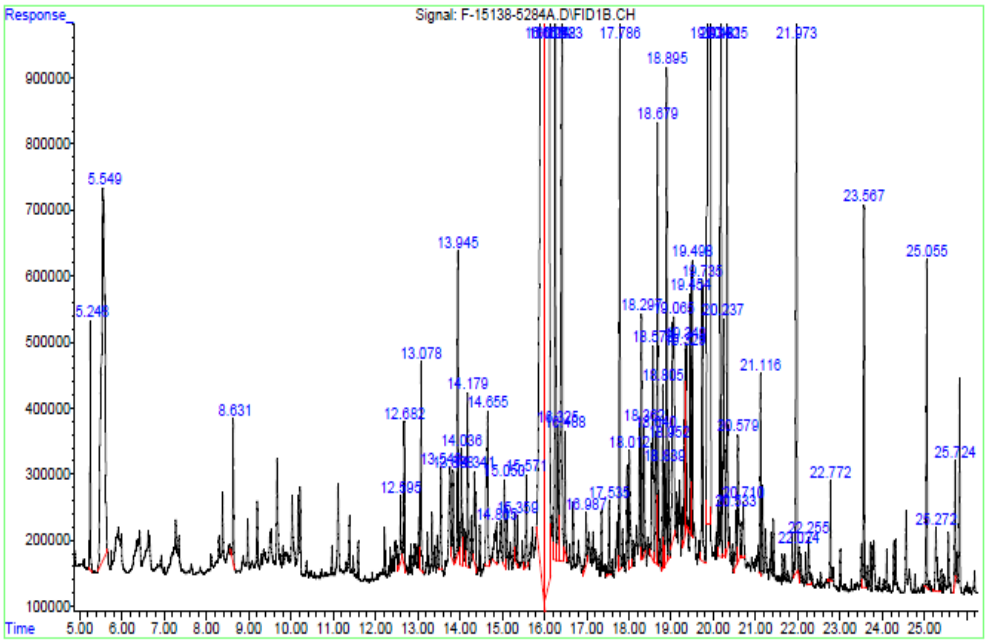

Figure 5: GC/FID profiles of *R. farinacea*

Table 1. Chemical composition of essential oil of *Evervia prunastri and Ramalina farinacea*

	KI	*E. prunastri*	*R. farinacea*		KI	*E. prunastri*	*R. farinacea*
Total		93.64	76.04	Total		93.64	76.04
Number of compound		32	34	Number of compound		32	34
Yield %		0.01	0.01	Yield %		0.01	0.01
α-pinene	940	0.66	**4.04**	Cedrol-epi	1675	0.37	0.00
β-pinene	984	0.28	0.59	Cubenol-1,10 Diepi	1675	0.00	0.87
Myrcene	996	0.00	0.79	n-tetradecanol	1683	**8.27**	**7.68**
Cymene-ortho	1032	0.00	1.65	Heptadecane (C17)	1713	**4.70**	0.00
Limonene	1037	0.00	1.09	Tridecenol acetate	1729	**2.05**	0.00
β-phellandrene	1038	0.00	0.03	Pentadecanol-n	1807	0.68	0.00
Linalool	1108	0.00	**2.43**	Octadecane (C18)	1813	0.84	0.00
n-nonanal	1113	0.29	0.00	Isopropyl tetradecanoate	1837	0.78	0.00
Borneol	1184	0.00	0.38	n-hexadecanol	1907	**2.32**	0.00
α-terpineol	1206	0.00	1.23	Nonadecane C19	1915	1.83	0.00
Cogeijerene	1254	0.00	1.14	7-hexadecen-16-olide-Z	1935	0.80	0.00
Bornyl acetate	1295	0.00	0.43	Acetoxyeudesman 4-α-ol-11	1973	0.55	0.00
Undecanone-2	1302	0.68	0.29	Eicosene-1	2015	**3.82**	**8.26**
Tridecane	1309	0.28	0.00	Manool oxide-13-epi	2020	0.00	**5.40**
Undecanal	1319	0.51	0.00	Kaurene	2025	0.00	0.42
Tetradecane (C14)	1410	0.60	0.00	Manool	2077	0.49	**14.21**
Tridecanone-2	1413	0.49	0.00	n-octadecanol	2106	**54.06**	**10.24**
Cymene-2.5-d-methoxy	1423	0.00	0.52	Docosene-1 (C22)	2154	0.40	0.74
Crowceacin	1446	0.00	**2.54**	Docosane	2217	0.69	1.47
Germacrene D	1497	0.00	0.63	Octadecanol acetate	2224	0.57	0.00
Pentadecane	1511	1.41	0.00	Abietal dehydro	2292	0.00	0.92
Tridecanal	1524	0.35	0.00	Tricosane	2317	0.53	0.48
Nerolidol-E	1574	0.00	0.71	Abietal	2340	0.00	**3.75**
Ionone dimethyl	1582	0.00	0.88	Tetracosane	2418	0.52	0.93
Caryophyllene oxide	1601	0.00	0.80	Pentacosane	2518	0.65	0.51
Hexadecane (C16)	1612	1.46	0.00	Hexacosane	2620	0.25	0.00
Tetradecanal	1626	0.97	0.00	Heptacosane	2721	0.49	0.00

Table 2. Antibacterial activity of essential oil extracts of lichens

Bacteria	G	*E. prunastri*			*R. farinacea*		
		Dilution					
		1	1/2	1/5	1	1/2	1/5
Staphylococcus aureus ATCC 25923	25	7	7	0	7	0	0
Staphylococcus epidermidis ATCC 12228	23	9	7	8	8	7	0
Escherichia coli ATCC 25922	14	7	0	0	8	7	0
Pseudomonas aerugenosa ATCC 27853	26	12	9	8	10	7	0
G = Gentamicine; ATCC: American Type Culture Collection; Inhibition zone (diameter of the disk, 6 mm, included), values represent average of 3 determinations;							

tetradecanol (8.27-7.68%) and eicosene-1 (3.82-8.26%) respectively.

E. Prunastri is isolated by the presence of heptadecane (C17) (4.70%), hexadecanol (2.32%) and tridecenol acetate (2.05%), while *R. farinacea* is characterized by presence of manool (14.21%), manool epi-13-oxide (5.40%), α-pinene (4.04%), Abietal (3.75%) and crowceacin (2.54%).

The anti-bacterial activity of the essential oils was determined by the disc diffusion method. The gram negative and gram positive bacteria strains used in this study showed a few significant resistances to the essential oil of *E. prunastri and R. farinacea* (Table 2). The inhibitions zones of *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 is less than 1/3 (7-9 mm) in the activity of gentamicin (23-25 mm), indicating that the effect of *E. prunastri* oil on these bacteria is very low.

The inhibitions zones of *E. coli* ATCC 25922 and *P. aerugenosa* ATCC 27853, represent half (7-12 mm) of the gentamicin activity (14-26 mm), indicating that the effect of oil *R. farinacea* on the bacteria gram-negative is average.

DISCUSSION

In the present study, the tested lichen extracts showed relatively a very low antimicrobial activity. The intensity of the antimicrobial effect depended on the species of lichen, the method of compounds extract, its concentration and the tested organism. The extracted with acetone, chloroform, diethyl ether and methanol, showed the presence of significant antimicrobial activity against several bacteria and fungi (Candan et al*.,* 2007).

The hexane extract of *Evernia prunastri* had a low antimicrobial activity among the species tested (Aslan et al., 2006; Rankovic et al., 2010). The acetone extracts of the tree species of *Parmelia* were examined, they showed a strong antimicrobial and cytotoxic activities (Kosanic et al*.,* 2011). The differences in antimicrobial activity of different species of lichens studied are probably a consequence of the presence of different components with antimicrobial activity (Aslan et al*.,* 2006; Adedapo et al*.,* 2008; Rankovic et al., 2010; Kosanic et al*.,* 2011).

Conclusion

It can be stated that the tested lichen extracts have a low antimicrobial activities in vitro. These lichens appear to be bad antimicrobial agents and could also be probably irrelevant for the food industry and in the control of various human, animal and plant diseases. Further studies should be done to search for new compounds from lichens that exhibit strong antimicrobial, antioxidant and anticancer activities.

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Competing interests

The authors declare that they have no competing interests

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