

EVALUATION OF THE ANTIMICROBIAL PROPERTIES OF SOME LICHENS

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Abstract: *In vitro* antimicrobial activities of the acetone and the chloroform extracts of the lichens, *Alectoria sarmentosa* (Ach.) Ach., *Bryoria fuscescens* (Gyeln.) Brodo and *D.Hawksw*, *Evernia divaricata* (L.) Ach., *Platismatia glauca* (L.) W.L. Culb. and C.F. Culb. and *Ramalina farinacea* (L.) Ach., against three gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* ATCC 11666 and *Acinetobacter* sp.) were investigated by using the paper disc method. All extracts were found to possess antimicrobial activity, *A. sarmentosa* having the best antibacterial properties.

Key words: lichen, antimicrobial activity

INTRODUCTION

Lichens are well-known symbiotic associations between fungi and algae, so called "lichenized fungi", including over 20000 species all over the world. These unique organisms are able to produce lichen-specific secondary compounds which have been used in medicine, food, cosmetics, dye and for other ethnobotanical purposes in popular and modern aspects from ancient to recent times (Çobanoğlu & Yavuz 2003, Çobanoğlu et al. 2004, Llano 1950, Romagni & Dayan 2002). Many species have antimicrobial, antifungal, antiviral, anticancer and anti-inflammatory effects supported by scientific evidence (Ingolfsdottir 2002, Romagni & Dayan 2002).

Lichens are represented in Turkey by approximately 2000 recorded taxa, and many species have been included in the studies on biological, such as antimicrobial and antifungal activities (Aslan et al. 2001, 2006, Candan et al. 2007, Çobanoğlu et al. 2006, Güllüce et al. 2006, Tay et al. 2006) antioxidant activities (Aslan et al. 2006, Güllüce et al. 2006) and antiviral activities (Karagöz & Aslan 2005).

In this study, antimicrobial properties of five lichens, foliose and fruticose species according to their morphological character, are presented. These species have potential medicinal and economical value due to their secondary substances (Romagni & Dayan 2002). The lichen species tested for the antimicrobial activities are epiphytic species with wide distribution in Turkey, *Ramalina farinacea*, *Platismatia glauca*, *Evernia divaricata*, *Bryoria fuscescens* and *Alectoria sarmentosa*. Their chemical constituents and the areas for potential uses are summarized in the Table 1, based on the data in Purvis et al. (1992) and Romagni & Dayan (2002).

Table 1.
The secondary compounds and potential uses of the tested lichen species.

Lichen species	Secondary metabolites	Potential uses
<i>Alectoria sarmentosa</i>	Alectosarmentin, dibenzofuran, usnic acid, alectoronic acid, depsidones	Anti-inflammatory, anticancer, perfume industry, antimicrobial, cosmetic, fungicidal, herbicidal
<i>Ramalina farinacea</i>	Obtusatic acid, paradeside, depsidones, usnic acid	Anticancer, Anti-inflammatory, inhibitor of prostaglandin synthesis, antimicrobial, cosmetic, fungicidal,
<i>Platismatia glauca</i>	Caperatic acid, atranorin	Anticancer, fungitoxic, perfume industry, ethnobotanical
<i>Evernia divaricata</i>	Divaricatic acid, evernic acid, paradeside	Perfume industry
<i>Bryoria fuscescens</i>	Fumarprotocetric acid, atranorin	Anticancer, fungitoxic, perfume industry, ethnobotanical

MATERIALS AND METHODS

Lichen Material and Test Microorganisms:

Since lichens grow very slowly (about 1 mm /year) in nature and are not amenable to culture practice, species with wider distribution and larger thalli should be selected in order to be able to harvest sufficient amount of thallus material. Lichen samples collected in the province of Bolu, abundantly found in Şerif Yüksel Research Forest, in July 2005, were used for the present antibacterial tests. *Alectoria sarmentosa* (Ach.) Ach., *Bryoria fuscescens* (Gyeln.) Brodo and D.Hawksw, *Evernia divaricata* (L.) Ach. and *Ramalina farinacea* (L.) Ach. are fruticose species and *Platismatia glauca* (L.) W.L. Culb. and C.F. Culb. is a foliose species.

The test microorganisms, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Escherichia coli* ATCC 11666, were provided by the University of Istanbul, Faculty of Cerrahpaşa Medicine, Microbiology Department.

Extract Preparations:

The air-dried samples were ground by means of mortar and pestle. Samples (3 g) of powdered lichen material were successively extracted in a Soxhlet apparatus by using 270 ml of chloroform and acetone, respectively. Extracts were evaporated to dryness under reduced pressure. The extracts were sterilized by membrane filtration using 0.2 µm pore size Millipore filters and were kept at 4°C until assay.

Preparation of Discs and Cultures of Microorganisms:

The disc diffusion susceptibility test was used to screen susceptibility of gram negative bacteria to the lichen extracts. The bacterial test strains were grown in nutrient broth until 0.5 Mc Farland standards and plated onto nutrient agar plates (Difco). The test solutions were screened by adding 20 µl of the aqueous suspensions onto filter paper discs (6 mm diameter Whatman paper discs) allowing the solvent to evaporate between applications.

Determination of Antibacterial Activity:

The filter paper discs were placed on to the test agar inoculated with the test strains. The plates were incubated at 37°C overnight under microaerophilic conditions. Test plates were observed under the stereomicroscope and then zones of bacterial growth inhibition were measured (Bauer et al. 1966), and are indicated in Table 2. All experiments were done in triplicate and checked with the control plates.

Table 2.
Inhibition Zone Measurements (Diameters; mm) of the Bacterial Growth.

Lichen species	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter</i> sp.	<i>Escherichia coli</i> ATCC 11666
<i>Alectoria sarmentosa</i> A*/C*	10.30 / 10.20	9.50 / -	10.10 / 10.10
<i>Ramalina farinacea</i> A*/C*	10.20 / -	10.30 / -	- / 8.00
<i>Platismatia glauca</i> A*/C*	- / 10.20	9.00 / -	10.1 / 8.00
<i>Evernia divaricata</i> A*/C*	8.00 / -	- / 8.00	10.25 / 10.15
<i>Bryoria fuscescens</i> A*/C*	10.20 / 10.25	8.00 / 8.50	10.30 / -
Control A*/C*	- / -	- / -	- / -

A*/ C* : Acetone/Chloroform [-]: No inhibition

RESULTS

The antimicrobial activity of the extracts of *Ramalina farinacea*, *Platismatia glauca*, *Evernia divaricata*, *Bryoria fuscescens* and *Alectoria sarmentosa*

against three gram negative bacteria were investigated. The diameters of bacterial growth inhibition zones on *Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter* sp. were indicated in the Table 2. All five lichen species were found to have antibacterial activity against these bacteria to some extent. In addition, acetone as a solvent is more effective than chloroform according to our experimental results. The data in the present study not only confirmed the previous evidences of presence of antibiotic substances in the extracts of some lichen species, but also reported antimicrobial activities of *A. sarmentosa*, *B. fuscescens* and *P. glauca*, for the first time.

DISCUSSION

The acetone extract of *A. sarmentosa* indicated the highest activity on *P. aeruginosa* and the lowest activity on *Acinetobacter* sp., while its chloroform extract was not active. The acetone extract of the lichen *R. farinacea* showed activity on *Acinetobacter* sp. and *P. aeruginosa* but not against *E. coli*. Both extracts of *E. divaricata* showed higher activity against *E. coli* than the other two bacteria. The antimicrobial activity of *E. divaricata* against these bacteria corresponds with the results in Aslan et al. (2006). The acetone extracts of *B. fuscescens* showed the highest antimicrobial activity on *E. coli* than the other lichen species. Antimicrobial activity of *P. glauca* was mentioned by Güllüce et al. (2006) but against different bacteria.

Tay et al. (2004) screened the antimicrobial activities of usnic acid, norstictic acid, protocetraric acid and acetone extracts of *R. farinacea*, and cited no inhibition for *E. coli* and *P. aeruginosa*. It was emphasized that (+)-usnic acid showed antimicrobial activity against the same microorganisms as the acetone extract did and it was concluded that (+)-usnic acid was probably the major antimicrobial agent in *R. farinacea*. Usnic acid has been known for a long time as a very important lichen substance due to its high biological activity in various aspects. In the review of Ingolfssdottir (2002), the antimicrobial activity of natural isomers of usnic acid, constituent of many lichen taxa such as *Alectoria*, *Cladonia*, *Lecanora*, *Evernia*, *Ramalina*, *Usnea*, against gram positive and gram negative bacteria were presented. No antimicrobial activity against *P. aeruginosa* and *E. coli* was exhibited. However, the ethanol, chloroform and *n*-hexane extracts of *R. farinacea* was found active by Esimone & Adikwu (1999) against *E. coli* and *P. aeruginosa*. Antibacterial activity against these two bacteria was observed in the study of Kekuda et al. (2009), comparing combination of

extracts of *Ramalina hossei* and *Parmotrema pseudotinctorum* with or without honey. We also found *R. farinacea* active against the both bacteria. The reason for different results may be due to different applications of the experimental procedure.

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