Comparative Study Between Antioxidant Activity and Antibacterial Effect of Usnea barbata (L)F.H.Wigg Extracts and Volatile Oils Marked in Romania

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Abstract

Usnea barbata (L.)F.H.Wigg is a lichen used in traditional medicine about 2000 years. Responsible for it biological effects are the secondary metabolites. Essential oils are used in aromatherapy because of the antimicrobial activity of their compounds. The aim of this study is to establish if it is a correlation between the antioxidant activity and antibacterial effect of both types of natural products.. In this study were used two Usnea barbata extracts and five essential oils – aetheroleum (aeth.) - from the same producer: Thymi aeth., Melissae aeth., Menthae aeth., Lavandulae aeth. and Eucalypti aeth. The antioxidant activity was determined by spectrophotometric method. The obtained results showed that all the analyzed Usnea barbata extracts and essential oils have antioxidant potential, with various intensities. Correlation of its antioxidant activity with antibacterial effects, shows that between both mentioned actions there is a relationship of direct proportionality.

Key words: Usnea barbata (L.)F.H.Wigg extracts, essential oils, antioxidant activity, antibacterial effect
J.E.L. classification: I10

1. Introduction

In the last years, there has been a tendency to supplement the action of synthetic drugs with natural remedies, given the fact that synthetic drugs have, in addition to therapeutic benefits, multiple side effects (Newman and Cragg, 2016).

A problem of global medical interest is the emergence of resistance of pathogenic bacteria to common antibiotics (Aslam *et al.*, 2018) this problem required extensive research to discover new structures with antibacterial effect. To this end, complex teams of researchers study the mechanisms of antibiotic resistance, while pursuing new ways to obtain alternative natural remedies with antibacterial action (Ventola, 2015)

Another remarkable aspect is that many species of plants have antioxidant properties (Salehi *et al.*, 2018), especially due to the content of polyphenols; they play an important role, both in the normal growth and development of plants, and in optimizing the defense abilities against oxidative stress (Jamshidi-Kia *et al.*, 2020) Oxidative stress, induced by a number of environmental factors (pollution, ultraviolet radiation, various pathogenic microorganisms), food and drugs, is involved in the generation and evolution of more than 200 severe diseases (Li *et al.*, 2015) including: cardiovascular disease (Dubois-deruy *et al.*, 2020), periodontal disease (Wang, Andrukhov and Rausch-Fan, 2017) and various forms of cancer (Aggarwal *et al.*, 2019).

In this context, plants are a valuable source of biologically active natural compounds, and research is aimed at their use in antibacterial and antioxidant therapy (Alscher and Hess, 2017). Isolated constituents may be useful as alternative therapeutic agents or as core nuclei for new synthetic products with increased activity and / or reduced toxicity . Lichens are also fall into the category of plants that have remarkable antibacterial and antioxidant properties (Shrestha and St. Clair, 2013) .They are a unique group in the plant world and, at the same time, are the most widespread symbiotic organisms in nature, inhabiting more than 8% of the earth's surface (Fernández-Moriano, Gómez-Serranillos and Crespo, 2016).Also, numerous medicinal plants are studied for the essential oils content, with many biological actions (Tohidi, Rahimmalek and Trindade, 2019) (Vergis *et al.*, 2015)

The aim of this work is to analyze some Usnea barbata L. lichen extracts and some essential oils for its antioxidant activity and antibacterial effect, trying to make a correlation between this two actions.

2. Theoretical background

By the UHPLC-ESI-OT-MS-MS technique, in Usnea barbata (L.) F.H.Wigg. the following groups of secondary metabolites have been isolated (Salgado *et al.*, 2017):

depsides: thamnolic acid, haemathamnolic acid, gryophoric acid, lecanoric acid, diffractaic acid, barbatic acid, methyl-8-hydroxy-4-O-divaricatic acid, sekikaic acid, barbatolic acid, 8-hydroxybarbatic acid, chloroatranorin and atranorin;

depsidones: salazinic acid, siphulellic acid, α-acetylconstictic acid, galbanic acid, norstictic acid, stictic acid, cronorstictic acid, lobaric acid;

lipids: tetrahydroxydocosanoic acid tetrahydroxytricosanoic acid, , tetrahydroxyhexacosanoic acid, nonahydroxyoctacosanoic, tetrahydroxyyeicosanoic acid, heptahydroxytricosatrienoic acid, tetrahydroxydocosanoic acid;

diphenyl ethers: β-alectoronic acid;

dibenzofurans: usnic acid.

The therapeutical aplications of Usnea barbata (L.) F.H.Wigg. is based on the history of its use in traditional medicine, extensive phytochemical research and pharmacological studies (Prateeksha *et al.*, 2016).Essential oils may contain numerous different organic compounds with a lot of biological activities (Astani and Schnitzler, 2014)(Kubatka *et al.*, 2020)(Contrucci *et al.*, 2019): phenolics, alcohols, oxides, ethers, esters, aldehydes, amides, ketones, amines, terpenes (Tohidi, Rahimmalek and Trindade, 2019).

3. Research methodology

3.1. Antioxidant activity assay

The antioxidant capacity was determined using the Jasco V630 UV-Vis spectrophotometer, by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, a standardized method for evaluating the ability of natural compounds to inactivate free radicals.

This method is performed by measuring the ability to neutralize DPPH radicals by the analyzed solutions; the purple color of the initial DPPH solution determinated at 515/517 nm is changed to light yellow as it appears in reduced form after the contact between DPPH solution and antioxidant compounds.

The absorption of 0.004% DPPH (Sigma-Aldrich, Mexico) in methanol was evaluated; this was brought into contact with the sample solutions and the absorbance was determined after 5 minutes; 2 Usnea barbata L. (UB) extracts solutions were made:

- S1 = UB extract from the dry lichen 200 mg / mL in 96% ethanol;
- S2 = UB extract 200 mg / mL in acetone;

For each UB extract: S1 and S2 (200mg / mL), the corresponding dilutions (100mg / mL) were made. DPPH free radical scavenger activity was calculated as follows:

% DPPH scavenger activity = 100 (Acontrol –Asample / Acontrol), where Acontrol and Asample are the absorbances determinated at 515 nm for the 0.004% DPPH solution in methanol and for the samples, 5 minutes after the addition of analyte solution (Popovici *et al.*, 2018).

Five essential oils with known antimicrobial properties (Reichling *et al.*, 2009), from the same producer were used: 1. Thyme essential oil (*Thymi aeth.*), 2. Lemon balm essential oil (*Melissae aeth.*), 3. Peppermint essential oil (*Menthae aeth.*), 4. Lavender essential oil (*Lavandulae aeth.*), 5. Eucalyptus essential oil (*Eucalypti aeth.*).

The antioxidant capacity of all the essential oils was determined by the same method; 2 mL of 0.5 mmol/L DPPH in methanol was mixed with 100 µL of different concentrations of each essential oil. After 20 min incubation, the absorbance (A) was measured at 517 nm with Jasco V630 UV-Vis spectrophotometer. The percentage of free radical-scavenging capacity was calculated with the same formula.

3.2. Antibacterial activity assay

Biological products taken from the oro-dental and pharyngeal cavity were studied: pharyngeal exudate, periodontal fluid, softened dentin from dental caries, nasal exudate and auricular secretion (the auricular canal and nasal fossae are close to the oral cavity). In auricular secretion was isolated Staphylococcus aureus, one of the most common bacterial species with high pathogenicity and recognized resistance to classical antibiotics.

Evaluation of antibacterial action was made by the *diffusimetric antibiogram method* (Hurezeanu *et al.*, 2013). The diffusimetric antibiogram method is based on the existence of a direct proportional relationship between the sensitivity of the tested bacteria and the size of the inhibition area of the bacterial colony developed around the antibiotic tablet.

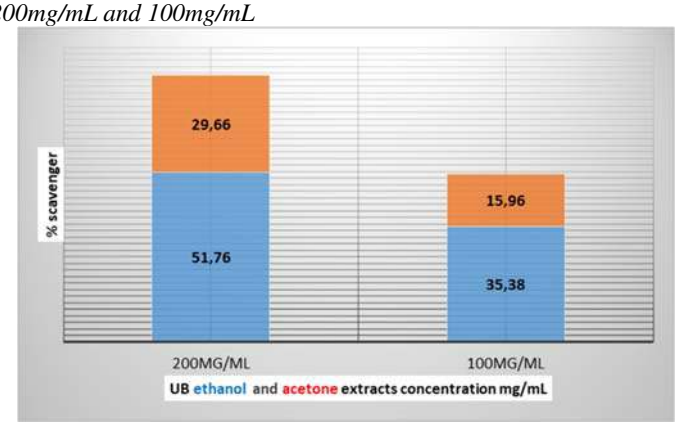
This standard method has been adapted to the specifics of the present study, replacing the antibiotic tablet with a filter paper disc with a diameter equal to its own, impregnated with the acetone and ethanol UB extracts, with 20% concentration value. Bacterial suspensions in 0,9% saline solution, with 0.5 Mac Farland turbidity, were uniformly seeded on the dry surfaces of Muller-Hinton culture media, and subsequently sterile filter paper discs were applied, saturated in the tested UB extracts. The amount of extract applied to each disc was 10 µL. Because that ethanol and acetone have their own antibacterial effects, after saturating the sterile filter paper discs with the corresponding extracts, the washers were left for 15 seconds to evaporate the solvent, before applying to the surface of the media with Staphylococcus aureus strain Subsequently, the Petri dishes were incubated for 24 hours at 37 o C, and at the end of this period the results were determinated.

4. Findings and discussions

4.1. Antioxidant activity assay

The obtained results show that UB extracts neutralized DPPH radicals with different degrees of scavenging activity (Figure 1). It was observed that the UB ethanol extract has the highest antioxidant activity.

Figure no. 1. Antioxidant activity of UB ethanol (marked with blue color) and acetone (marked with red color) extracts, 200mg/mL and 100mg/mL

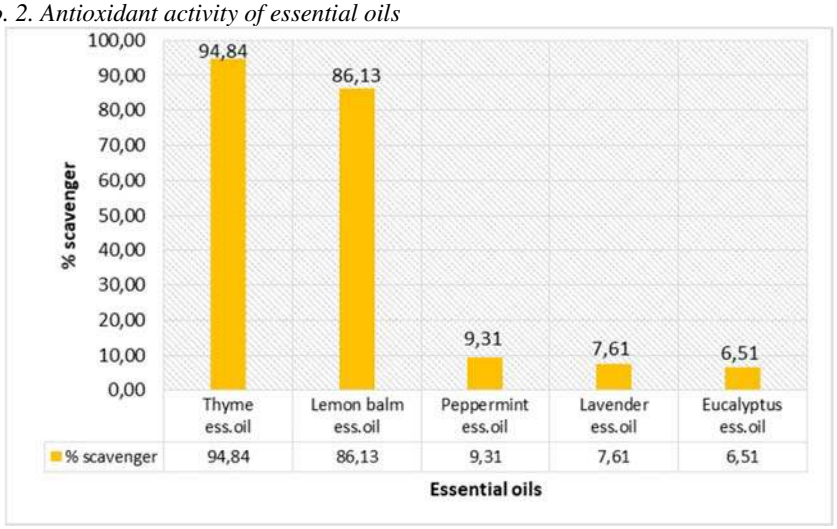


Source: authors' processing

The obtained results for the analysed essential oils (Figure 2) are the follows:

- *Thyme essential oil* and *lemon balm essential oil* have a DPPH free-radical scavenging activity values about 90%.
- *Pepermint essential oil, lavender essential oil* and *eucalyptus essential oil* have a DPPH free-radical scavenging activity values under 10%, acting through menthol, linalool, respectively eucalyptol.

Figure no. 2. Antioxidant activity of essential oils

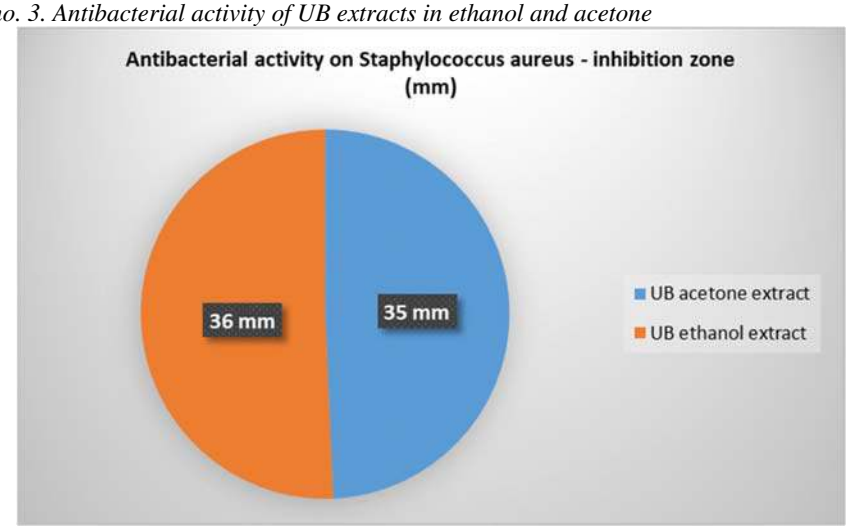


Source: authors' processing

4.2. Antibacterial activity assay

The results were quantified by measuring the diameter of the inhibition zones in millimeters, for each type of UB extract used: 36 mm for UB ethanol extract and 35 mm for UB acetone extract (Figure 3).

Figure no. 3. Antibacterial activity of UB extracts in ethanol and acetone



Source: authors' processing

The obtained results showed that the UB ethanol extract has a slightly higher antibacterial effect on Staphylococcus aureus, than UB acetone extract.

Correlating the antibacterial effect with the antioxidant potential for UB analyzed extracts, it can note that between both activities exist a direct proportionality.

For the five analyzed essential oils, there were correlated the antioxidant activities with antibacterial effects on Staphylococcus aureus found in the accessed scientific literature (Inouye, Takizawa and Yamaguchi, 2001). The antibacterial action of the 5 essential oils was quantified by minimal inhibitory dose (MID) of essential oil by gaseous contact, measured in mg/L air (Inouye, Takizawa and Yamaguchi, 2001); lower MID value means higher antibacterial action. The comparative data were presented in Table 1.

Table no. 1 Comparative analyzis between essential oils antioxidant activity (% scavenger) and antibacterial activity (MID mg/L air) on Staphylococcus aureus

Ess.oil	% scavenger	MID mg/L air
Thyme ess.oil	94,84	6,25
Lemon balm ess.oil	86,13	12,50
Peppermint ess.oil	9,31	25,00
Lavender ess.oil	7,61	50,00
Eucalyptus ess.oil	6,51	200,00

Source: adapted with the data from (Inouye, Takizawa and Yamaguchi, 2001)

Analyzing the data from Table 1 it can note that Thyme essential oil has the highest antioxidant activity and the best antibacterial effect (the smallest MID value). The antioxidant potential decrease in order: Thyme essential oil, Lemon balm essential oil, Peppermint essential oil, Lavender essential oil, and, on the last place, Eucalyptus essential oils (Figure 2), like the antibacterial action, quantified by MID value (Inouye, Takizawa and Yamaguchi, 2001).

5. Conclusions

This study showed the direct proportionality between the antioxidant activity and antibacterial effect of the UB extracts and essential oils.

Analysis of all the actions of this studied natural products correlated with results of this work, can suggest that these natural products can be used as complementary therapy for increase the immune defense, by the antioxidant activity and for the treatment of staphylococcal infections.

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