COMPARATIVE STUDY REGARDING ANTIBACTERIAL ACTION OF THE *USNEA BARBATA* L. EXTRACTS ON GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA FROM THE ORO-DENTAL CAVITY

PhD Student Violeta Popovici[1]
Assoc. Prof. Dr. Laura Bucur[2]
Assoc. Prof. Dr. Antoanela Popescu [2]
Prof. Dr. AurelianaCaraiane [1]
Prof. Dr. Victoria Badea [1]
[1]Ovidius University, Faculty of Dental Medicine, Constanta, Romania
[2]Ovidius University, Faculty of Pharmacy, Constanta, Romania

ABSTRACT

This study is based on the hypothesis that various extracts from *Usnea barbata* L. have antibacterial action. Six lichen extracts of different concentrations in: 96% ethanol, acetone and water, have been studied. Their inhibitory action was tested by adapting a diffusimetric antibiogram, made for three pathogenic Gram-positive and Gram-negative bacteria from the oro-dental cavity: *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*

Our results showed that on *Staphylococcus aureus,* acetone and ethanolic *Usnea barbata* L. extracts have a great antibacterial action. On Gram-negative bacteria, in the zone of inhibition of the acetone and ethanolic extracts from *Usnea barbata* L., the development of resistant mutant strains was observed. On all the studied pathogenic bacteria: aqueous extract from *Usnea barbata* L. is totally inactive.

In conclusion, in oro-dental infections where strains of *Staphylococcus* are involved, acetone and ethanolic *Usnea barbata* L. extracts could be very effective as auxiliary treatment, together with classic antibiotics.

Keywords: Usnea barbata L. extracts, antibacterial action, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli

AIM

In this work, we proposed to present, as result of our research, the antibacterial action of different extracts from *Usnea barbata* L., from North-Eastern Carpathian Mountains, Romania. Our study tested this action on Gram-positive and Gram-negative bacteria, from an oro-dental cavity.

INTRODUCTION

Healthcare research refers, in a great measure, to find natural sources of bioactive compounds for the treatment of various diseases.

Therapeutic practice using active substances biosynthesized with the help of plants defines phytotherapy, an old branch of contemporary therapeutics, with a range and tradition from ancient times [2], [6], [11]. The vegetal products are indicated in chronic diseases as well as in acute disorders [8], [11].

Currently, clinicians around the world are facing a serious problem of increasing bacterial resistance to antibiotics [1], [4], [6], [12]. One of the most important research goals related to bacterial resistance is represented by the discovery of new compounds with new structures and new mechanisms of action, which will prevent the occurrence and development of bacteria resistance to antibiotics [4].

Usnea barbata L. is a lichen, with a large habitat on Earth; it is used in traditional chinese medicine, from 101 before Hr.[10], [11]. A lichen is a simbiont organism; the symbiosis is realized between a fungus (*Ascomycetes* or *Bazidiomycetes*) and algae or cyanobacteria, who grow together (*Figures 1, 2*) [5], [10], [11], [12].

Figure 1. Usnea barbata L.

Figure 2. Usneae lichen – vegetal product

The most important natural compounds from *Usnea* spp are secondary metabolits, especially usnic acid and polyphenols; usnic acid is a complex dibenzofuran derivative. It has many pharmacological activities: antibacterial, antifungal, antiviral, antioxidant, antiinflamatory, analgesic-antipyretic, anti-cancer, genotoxic, antigenotoxic, antimutagenic, antiplatelet/anti-thrombotic, anti-ulcerogenic (*Figure 3*) [2], [10].

The polyphenols (including: gallic acid, chlorogenic acid, p-coumaric acid) have a strong antioxidant activity [1], [4], [11].

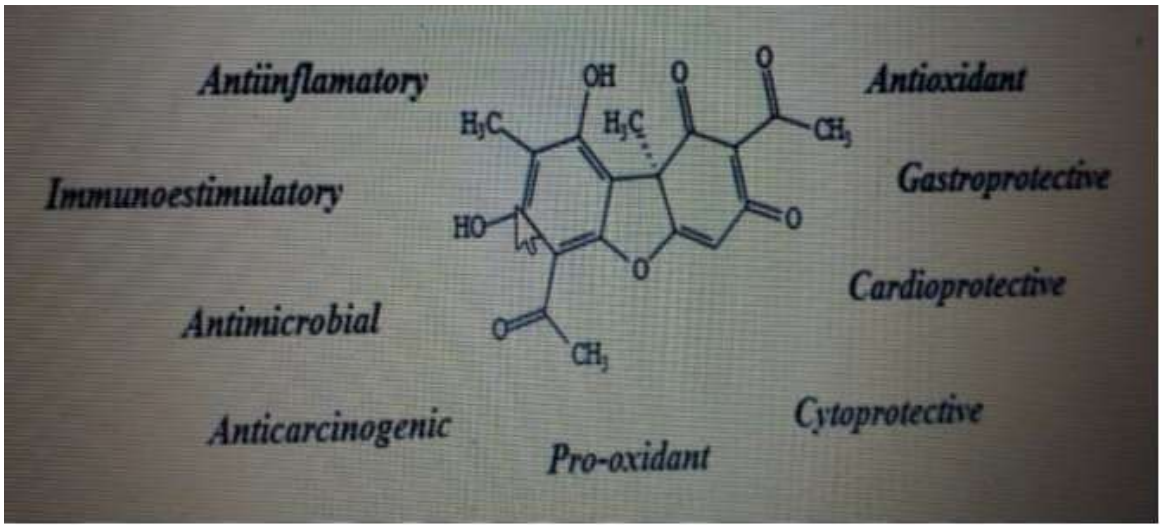

Figure 3. Usnic acid: chemical structure and pharmacological action

MATERIALS AND METHODS

Obtaining vegetal product *Usneae lichen:*

Usneae lichen is the *Usnea barbata* L. dry *thallus* which was harvested from Călimani Mountains, Romania, in the time of early spring, when the lichen has the highest content in bioactive compounds [5]. Every *thallus* was harvested by hand, one by one; the lichen was cleaned and dried at a constant temperature below 25° C in an airy room, sheltered from the sun's rays; finally, the herbal material is preserved in the same conditions. The vegetal product taken into work for this purpose was represented by the *thallus* of lichen *Usnea barbata* L. dried and brought to the degree of crushing required for loose tissues (*Figure 2*) [5].

Obtaining *Usneae lichen* extracts:

Six extractive solutions from *Usnea barbata L*. were taken into our study: three extracts (5% si 20%) in acetone, two extracts (5% si 20%) in 96% ethanol and one extract (20%) in water. The extracts were prepared as follows: for each extract were taken the appropriate amount of vegetal product depending on the concentration of each extract . Thus, three extracts were obtained in three different solvents: water, acetone and 96% ethanol [10].

Acetone and 96% ethanolic extracts were obtained by cold maceration for seven days; aqueous extract was obtained fresh by hot reflux for 1 hour. The three resulting extractive solutions were filtered and then made up to 100 mL volumetric flask with each solvent used in the extraction (*Figure 4, 5*). [10].

The six *Usneae lichen* extractive solutions was: S_1, *20% acetone extract*, S_2, *5% acetone extract*, S_4, *20% ethanolic extract*, S_6, *20% aqueous extract*. S_3, *5% acetone extract* and S_5, 5% *ethanolic extract,* were prepared 6 months before and used in all the preliminary determinations [10].

Figure 4, 5: Usneae lichen extracts: ethanolic, acetone and aqueous

Microbiological studies

The research was made on Gram-positive and Gram-negative bacterial strains isolated from oro-dental cavity: *Staphylococcus aureus, Esherichia coli* and *Pseudomonas aeruginosa.* The antimicrobial activity was determined by the diffusimetric antibiogram method [1], [3], [4], [6], [7], [8], [9], [12], [13]. The principle of the method involves a strict relationship between the level of susceptibility and the size of the inhibition area of the germ colony development around the filter paper disc, impregnated with the test solution. Microorganism-suspension test: in the isotonic sodium chloride solution, suspensions have tube's turbidity of 0.5 Mac Farland [4]. Microorganism-suspension tests were inoculated on the culture media Muller-Hinton agar [1], [4]. On the surface of the media, discs of sterile filter paper were placed, soaked with 10 mcL of the test solutions.

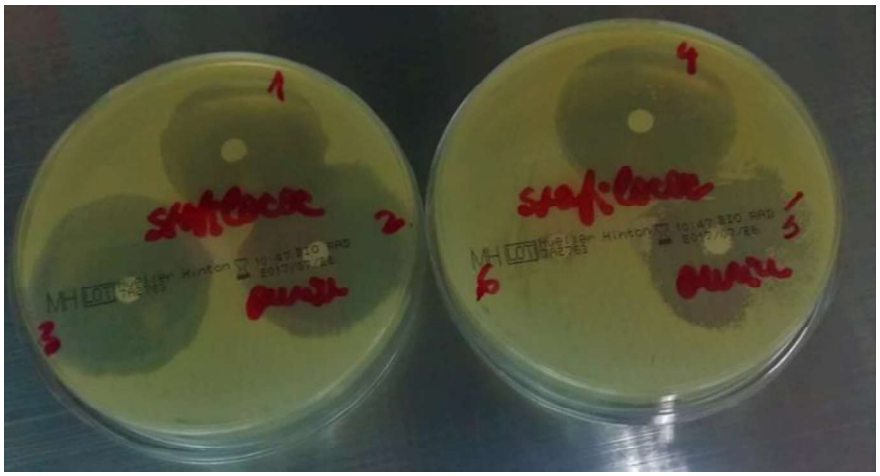

Figure 6. The technique of the diffusimetric antibiogram method - the placement of the discs of sterile filter paper and the marking of the Petri dishes.

The Petri dishes have then been incubated at 37°C, 24 hours for all bacteria [4]. After that we examined the Petri dishes, by recording the diameter of the inhibition zones for each microorganism and for each dilution of the test sample [1], [4].

RESULTS AND DISCUSSIONS

Results: The diameter of the inhibition zone was measured for each extract and dilution and was expressed in millimetres (*Table 1*).

In *Table 1*, S_1-S_6 are follows: S_1, 20% acetone extract, S_2, 5% acetone extract, S_4, 20% ethanolic extract, S_6, 20% aqueous extract. S_3, 5% acetone extract and S_5, 5% ethanolic extract, were prepared 6 months before and used in all the preliminary determinations.

Table 1. The diameters of the inhibition zones on bacterial cultures for S_1-S_6 samples:

SAMPLE	STUDIED BACTERIA SPECIES		
	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
	DIAMETER OF INHIBITION ZONES (mm)		
S_1	35 mm	22 mm	26 mm
S_2	35 mm	22 mm	26 mm
S_3	40 mm	25 mm	35 mm
S_4	36 mm	38 mm	40 mm
S_5	36 mm	32 mm	40 mm
S_6	0 mm	0 mm	0 mm

By using the diffusimetric antibiogram method, our results showed that the tested solutions have different antibacterial activity (*Figures 7, 8, 9*).

On *Staphylococcus aureus,* acetone and ethanolic *Usneae lichen* extracts have a great inhibitory activity; the ethanolic extracts are more active than the acetone ones (*Figure 6).*

On Gram-negative bacteria, in the zone of inhibition of the acetone and ethanolic extracts from *Usnea barbata* L., development of resistant mutant strains was observed. This result was observed on both studied Gram-negative bacteria; it was more intense at *Escherichia coli* than at *Pseudomonas aeruginosa.* This effect was stronger for the acetone extracts than for the ethanolic ones, taken in our study (*Figures 8, 9).*

On all the studied pathogenic bacteria*: Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli,* aqueous extract from *Usnea barbata* L. is totally inactive *(Figures 7, 8, 9).*

Figure 7. The inhibitory activity of Usnea barbata L. extracts on Staphylococcus aureus

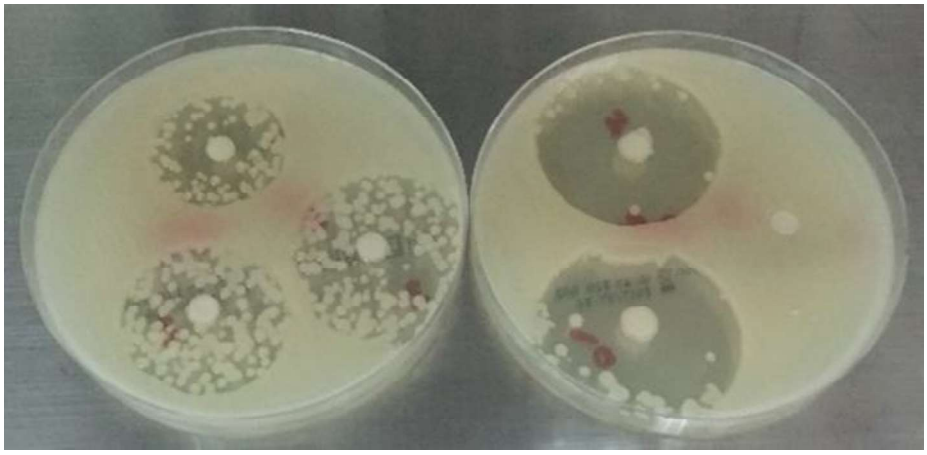

Figure 8. The inhibitory activity of Usnea barbata L. extracts on Escherichia coli

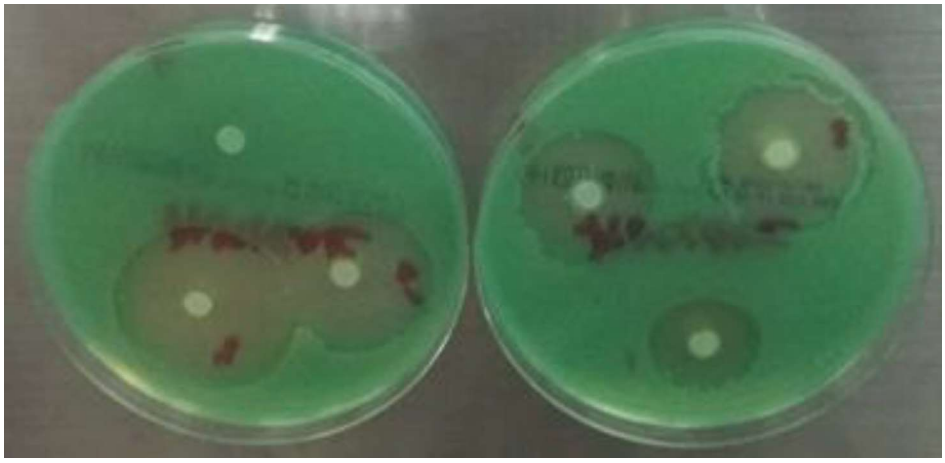

Figure 9. The inhibitory activity of Usnea barbata L.extracts on Pseudomonas aeruginosa

Discussions:

The highest level of sensitivity is recorded at *Staphylococcus aureus* (as Gram-positive bacteria) and *Pseudomonas aeruginosa* (as Gram-negative bacteria).

Comparing the Gram-negative bacteria, we can note that although it is a bacterial species with significant resistance to antibacterial agents, *Pseudomonas aeruginosa* has recorded higher sensitivity levels than *Escherichia coli* in the acetone and ethanolic extracts tested (Table 1). In support of this finding, it is particularly significant that the presence of resistant mutants has been observed in *Escherichia coli* inhibition areas (Figure 8).

The team of researchers who performed this present study, previously identified and dosed the secondary metabolites, usnic acid and polyphenols, in various extracts of *Usnea barbata* L., by HPLC method; the results of their research have already been published [10]. Thus, the results of evaluating the antibacterial activity of the various extracts of *Usnea barbata* L. on Gram-positive bacteria, can be explained by the usnic acid and polyphenols content of each extractive solution of *Usnea barbata* L. taken into work [10].

In 2003, I.T. Madamombe and A.J. Afolayan, in their study about antibacterial action of *Usnea barbata* L. from South Africa, obtained the same results for this three bacteria; the same inhibitory action was showed by Cansaran et all in 2006 [2], [7].

CONCLUSION

It is obvious the great ability of the acetone and ethanolic *Usnea barbata* extracts to inhibit the growth of this one of the most pathogenic and frequently encountered Gram-positive bacteria.

This plant product may be used in oro-dental infections where strains of *Staphylococcus aureus* are involved, with great efficiency. It may be a valuable auxiliary treatment, completing classic antibiotic therapy in staphylococcal infections, resistant to common antibacterials.

On Gram-negative germs taken into study, we can observe that *Pseudomonas aeruginosa* had a high sensitivity to inhibitory activity of acetone and ethanolic extracts from *Usnea barbata* L.

ACKNOWLEDGEMENTS

In making this article, all the authors had equal contributions.

REFFERENCES

[1] Arsene A.L., Rodino S.,, Butu A., Petrache P., Iordache O., Butu M., Sudy on antimicrobial and antioxidant activity and phenolic content of ethanolic extract of *Humulus lupus*, Farmacia, 63(6): 851-857, 2015

[2] Cansaran D., Kahya D., Yurdakulol E., Atakol O., Identification and Quantitation of usnic acid from the Lichen *Usnea* species of Anatolia and Antimicrobial Activity, *Z. Naturforsch*, 61(11-12): 773-776, 2006

[3] Emrobowansan M. I., Masika P.I., Muchenje V., Falta D. Green E., In-vitro antibacterial sensitivity of *Usnea barbata* lichen extracted with methanol

and ethyl-acetate against selected *Staphylococcus* species from milk of cows with mastitis, Archiv Tierzucht 57, 25: 1-9, 2014

[4] Gegiu G.,Branza A., Bucur L., Grigorian M., Tache T., Badea V., Contribution to the antimicrobial and antifungal study of the aqueous extract of *Prunus spinosa* L. , Farmacia, 63 (2): 275-279 2015

[5] Hancianu M., Cioanca O., Aprotosoaie C., Miro A., Plante medicinale de la A la Z, Editura Polirom, Romania, 2014, 1260-1265

[6] Kamal S.,Manish S., Savita J., Assessment of Antibacterial Activity of *Usnea species* of Shimla Hills, Int.J.Curr.Microbiol.App.Sci , 4(7): 413-425, 2015

[7] Madamombe I.T.,Afolayan A.J., Evaluation of antimicrobial activity of extracts from South African *Usnea Barbata*, Pharmaceutical Biology, 41(3): 199-202, 2003

[8] Pavithra G.M., Vinayaka K.S. Rakesh K.N. , Syed Junaid , Dileep N., Prashith Kekuda T.R., Saba Siddiqua , Abhishiktha S. Naik, Antimicrobial and antioxidant activities of a macrolichen *Usnea pictoides* G. Awasthi (Parmeliaceae), Journal of Applied Pharmaceutical Science, 3: 154-160, 2013

[9] Pop C.E., Parvu M., Arsene L.A., Parvu A.E., Vodnar D.C., Tarcea M., Toiu A.M., Vlase L., Investigation of antioxidant and antimicrobial potential of some extracts from *Hedera helix* L., Farmacia, 65 (4): 624-629, 2017

[10] Popovici V., Bucur L., Popescu A., Caraiane A.,Badea V., Determination of the content in usnic acid and polyphenols from the extracts of *Usnea barbata* L and the evaluation of their antioxidant activity , Farmacia, , Vol. 66(2), 337-341, 2018

[11] Prateeksha B. S. †, Paliya R. †, Bajpai R., Jadaun V., Kumar J., Kumar S., Upreti D.K., Singh B.R.., Nayaka S., Joshi Y., Brahma N., The genus *Usnea*: a potent phytomedicine with multifarious ethnobotany phytochemistry and pharmacology - RSC Adv., 6: 21672–21696, 2016

[12] Srivastava P., Upreti D.K., Dhole T.N., Srivastava A.K., Nayak M.T., Antimicrobial Property of Extracts of Indian Lichen against Human Pathogenic Bacteria, Hindawi Publishing Corporation Interdisciplinary Perspectives on Infectious Diseases, Article ID 709348: 1-6, 2013

[13] Toiu A., Vlase L., Drăgoi C.M., Vodnard D., Oniga I., Phytochemical analysis, antioxidant and antibacterial activities of *Hypericum humifusum* L. (Hypericaceae). Farmacia, 64(5): 663-667A, 2016