"OVIDIUS" UNIVERSITY, CONSTANȚA

DOCTORAL SCHOOL OF MEDICINE

DOCTORAL AREA: DENTAL MEDICINE

Contributions regarding the study on antitumor and antibacterial activities of *Usnea barbata* (L.) F.H.Wigg. extracts in the oral and dental pathology

Abstract of PhD Thesis

PhD Coordinator

Professor Victoria Badea

PhD Student

Violeta Popovici

CONSTANȚA,

2019

"There is a means of expression accessible to all in the world:
it is the language of enthusiasm, of things done with love and perseverance
looking for something you want or you believe in ... "
(Paulo Coelho)

At this doctoral thesis I benefited from the guidance and collaboration of some people with remarkable professional and spiritual qualities, whom I would like to thank, with all my heart.

I am grateful for the privilege of having a PhD Coordinator on Mrs. Professor Victoria Badea, an professional elite and in the same time, a great personality; I heartedly thank her for the enthusiasm, openness and dedication with which she guided my steps in these studies realised during these years and for the warmth, sincerity and firmness with which she has supported me every time, in my spare moments.

I would like to thank to the referents, for the patience with which they analyzed the studies included in all the research reports that preceded this doctoral thesis.

Amazing thoughts of gratitude turn now to Mrs. Dean of the Faculty of Pharmacy in Constanța, Assoc.Prof. Laura-Adriana Bucur, who from the first year of doctoral studies guided me every step of the pharmacognostic research and who was with me "with and without time", during these years until final.
.

I send warm thanks to Miss Assoc. Prof. Antoanela Popescu, as well as to Assistant Prof. PhD. Verginica Schroder, for the studies realised together.

In the last year of doctoral studies I have benefited from two prestigious collaborations: the studies on tumor cell cultures were performed at the Institute of Biological Research Iași, and the UHPLC analysis of the dry lichen extract was performed at the Research Center for Instrumental Analysis "SCIENT" Bucharest.

Warm thanks, therefore, to Mrs. CEO Gabriela Vochița and to the team from the Institute of Biological Research Iasi, for the efforts made to purchase the CAL 27 (ATCC® CRL-2095 ™) tumor cell line, for the best collaboration during the studies on tumor cell cultures and for the prompt and efficient communication throughout this time.

I also thank to the team of the Research Center for Instrumental Analysis "SCIENT" Bucharest, for the availability and professionalism with which they collaborated for realise the UHPLC analysis of the extract used in the study of antitumor action.

I thank to Mr. Eugen Popa from Vatra Dornei who guided me in choosing of this species of lichen, as well as to Mrs. Assoc.Prof.PhD. Mariana Arcuș from the Department of Pharmaceutical Botany of the Faculty of Pharmacy Constanța for confirmation of the identity of this studied species.

Thanks for all the teachers who contributed to my professional training.

I would like to thank to my family, colleagues and to all those closed to me, who have been involved in realising to this doctoral research and have supported me during all the years of study.

Finally - but not in the least - my gratitude and thanksfull goes to the Archbishop Professor Teodosie Petrescu, for the confidence and the appreciation which he has always shown to me and for his unconditional, spiritual and material support, in all the efforts to realise this doctoral thesis.

CONTENTS

INTRODUCTION

In the recent years there has been a tendency to supplement the action of synthetic drugs with natural remedies, given that synthetic drugs have, besides the therapeutic benefits, multiple side effects and adverse effects [29, 45, 48, 59].

A problem of the world medical interest is the appearance of resistance of pathogenic bacteria to the usual antibiotics; this phenomenon required extensive research for the discovery of new antibacterial structures [48-72]. To this end, complex teams of researchers are studying the mechanisms of antibiotic resistance, while pursuing new ways of obtaining alternative natural remedies with antibacterial action [52, 85, 86, 116].

Another remarkable aspect is that many plant species have antioxidant properties, mainly due to the polyphenol content; they play an important role, both in the normal growth and development of plants, as well as in the optimization of the defense abilities against oxidative stress [30,180,181]. Oxidative stress is involved in the generation and development of more than 200 severe diseases, including: cardiovascular disease, Parkinson's disease, Alzheimer's disease, periodontal disease and various forms of cancer, including oropharyngeal cancer [172, 184].

The periodontal disease or the periodontitis is a multifactorial disease in which, the association between the action of the bacteria that make up the dental plate and the genetic factors is of particular importance in its later appearance and evolution.

The cancer is a heterogeneous class of disorders, characterized by uncontrolled cell division and their ability to invade nearby tissues and to metastasize, lymphatically or hematogenously, loco-regionally or remotely, into other tissues. and organs; it has an overall incidence estimated at 6 million cases per year, being the second major cause of death after cardiovascular disease [7]

The oro-maxillo-facial malignancies are generally characterized by: infiltrative-destructive tumor growth with local invasion and loco-regional and distant metastatic dissemination (with formation of cervical metastases, or in distant organs) [27, 28].

It is known that most of the synthetic chemotherapies currently used in antitumor therapy develop resistance over time and exhibit non-selective toxicity against normal cells; these side effects,

combined with adverse reactions and multiple drug interactions, are major drawbacks of chemotherapies [107-114, 169].

Therefore, obtaining of new antibacterial and antitumor drugs remains a major clinical challenge. In this context, the plants represent a valuable source of biologically active natural compounds, and the research is aimed at their use in anti-infectious and anti-cancer therapy [260, 261]; the isolated constituents may be useful as alternative therapeutic agents or, as basic nuclei for new synthetic products, with increased activity and / or reduced toxicity [10, 30, 169-172,].

In the category of plants that have remarkable antitumor, antibacterial and antioxidant properties, lichens are also found [5, 33-38, 45, 59, 111, 159, 160]. They are a unique group in the world of plants and, at the same time, they are the most widespread symbiotic organisms in nature, inhabiting more than 8% of the earth's surface of the earth [1,11, 13, 20, 26].

An important representative of the lichens is the genus *Usnea* Dill. ex. Adans., With over 350 species spread all over the globe; In the literature it is clear that many species of this genus have been used in traditional medicine for thousands of years in the treatment of various diseases [1, 26, 53].

Representatives of the genus *Usnea* Dill.ex Adans are also found in Romania [25, 228]; thus, starting from all the aforementioned considerations, the idea of realising this doctoral thesis was outlined, in which the antitumor and the antibacterial actions of the extracts of *Usnea barbata* (L.) F.H. Wigg., harvested from the Călimani mountains (900 m), Suceava county, which could be applicable in the treatment of oral and dental disorders.

The present doctoral thesis aims to study the therapeutic potential of the species *Usnea barbata* (L.) F.H.Wigg., making a personal contribution to the current data existing in the specialized literature.

GENERAL PART

The first chapter of the thesis describes the lichen species studied, *Usnea barbata* (L.) F.H.Wigg, mentioning its systematic classification.

A general presentation was made to the lichens, briefly presenting their morpho-physiological particularities, as a result of the symbiosis between a fungus and an alga / cyanobacterium; the genus

Usnea was then analyzed, describing at large the primary and secondary metabolites and their biological actions. Towards the end of the first part all these notions presented previously towards the characterization of the species Usnea barbata (L.) F.H.Wigg. Were concentrated, with the presentation of the anatomical-morphological and chemical properties, mentioning also the current phytotherapeutic applications.

In the second chapter the oxidative stress was presented, as a result of the overproduction of free radicals of oxygen (SRO), their role in generating a large systemic pathology was highlighted.

In the third chapter, the most common oro-maxillofacial disorders were presented: dental caries, periodontal disease and oro-maxillo-facial malignancies, emphasizing their etiology and treatment.

PERSONAL PART

The second part of the thesis is structured in 4 studies:

Usnea barbata (L.) F.H.Wigg - pharmacognostic analysis;

Determination of the cytotoxic properties of the *Usnea barbata* (L.) F.H.Wigg. dry acetone extract on *Artemia salina* L.;

Evaluation of the antitumor action of the *Usnea barbata* (L.) F.H.Wigg. dry acetone on the lingual carcinoma with CAL 27 squamous cells;

Determination of the antibacterial effects of the *Usnea barbata* (L.) F.H. Wigg extracts on the bacterial species isolated from oropharynx.

I. *Usnea barbata* (L.) F.H.Wigg. - pharmacognostic analysis

***Hypothesis of this study**:* the *Usnea barbata* (L.) F.H.Wigg extracts contain secondary metabolites that could be used in phytotherapy; this species could have a high content of phenolic compounds with antioxidant properties.

***Purpose of the study**:* obtaining of the *Usnea barbata* (L.) F.H.Wigg. extracts in different solvents, which allow the identification and quantitative determination of chemical constituents; evaluating the antioxidant activity of these extracts and highlighting the correlation between the polyphenolic content and their antioxidant potential.

I.1. Harvesting of the lichen material

The harvesting of the lichen material was realised in March 2016.

The *Usnea barbata* (L.) F.H.Wigg., habitat area is a dense forest of conifers (firs, spruce and pine) located in the high mountain area (Călimani mountains), at an altitude of about 900 m; the specific climate of these places is humid and cold.

The lichens are hanging on the branches and the trunk of the host tree, the most numerous and best developed representatives of the species are found at their peak, at heights of over 2 m; they were collected manually, directly from the conifer branches.

I.2. Obtaining of *Usneae lichen*

The freshly harvested plant material was cleaned by impurities and dried at constant temperature, below 250C, in an airy room, sheltered from the sun's rays. *Usneae lichen* is represented by the *Usnea barbata* (L.) F.H.Wigg. dry *thalli*. After drying, *Usneae lichen* was kept for a long time under the same conditions, in order to use it in the studies that followed.

I.3. Determination of the *Usneae lichen* identity

I.3.1. Macroscopic examination

Material and method

The material taken up was represented by *Usneae lichen.* The macroscopic characters of *Usneae lichen* were observed with the free eye, and its identification was made according to size, color, smell and taste. In the next stage *Usneae lichen* was ground and the peculiarities of the component elements were observed.

Results and discussions

Through the macroscopic examination, the *Usnea barbata* (L.) F.H.Wigg thalli was identified and its general properties were observed: the lichen *thalli* is light greenish-gray, soft, filiform, pendant, 15-20 cm long; it has dichotomous branches, with numerous branches that intersect, fixed by a darker gray opaque stalk. The smell of the dried plant is aromatic, specific, and the taste is bitter.

After evaluating all these morphological aspects, it was established that the harvested vegetable product belongs to *Usnea barbata* (L.) F.H. Wigg.; the fact is confirmed by the Department of Pharmaceutical Botany of the Faculty of Pharmacy of Constanța.

As a result of the grinding, two distinct components have been confirmed, which confirm the structure of a fruticose *thallus* mentioned in literature: a central cord, white-yellow, left intact after grinding and a part in the form of a gray-green powder. The central cord has the appearance of a thin yellow-white thread, which when wet, stretches like a rubber band.

I.3.2. Microscopic examination

Material and method

The parts obtained by grinding *Usneae lichen* (the central cord cut by scissors and powder) were analyzed under microscope.

Results and discussions

By microscopic examination, the symbiotic components of lichen, alga and fungus were identified: the inner part of the lichen - the central cord, homogeneous, consisting of compact mycelium hyphae and the outer part made of cyanophyte algae; existing chlorophyll in the algae gives the lichen its specific color, greenish-gray. In the first microscopic preparation, made from the central axis, the homogeneous composition, represented by the compact mycelium hyphae, was clearly observed; in the second microscopic preparation, made of powder, fragments of algae and rare mycelial hyphae were observed.

I.4. Determining the purity of the lichen product

The determination of the purity of a plant product comprises the following steps: determination of impurities in the same plant and determination of foreign bodies of the producing plant [233].

Results and discussions

According to the provisions of FR X, the product showed no impurities of any kind, so it can be considered 100% pure [234]. This result is very important for assessing the quality of *Usnea barbata* (L.) F.H.Wigg. harvested, because the relevance of the subsequent determinations depends very much by the purity of the *Usneae lichen* analyzed.

I.5. Determination of drying loss for *Usnea barbata* (L.) F.H.Wigg

Material and method

The material was represented by the crushed *Usneae lichen;* the used method is provided in the Romanian Pharmacopoeia X Edition (FR X) and in the European Pharmacopoeia 9.0. (EP 9). [234, 235].

Results and discussions

The drying loss (PPU) calculated according to the Romanian Pharmacopoeia X edition is a very useful parameter in the quantitative determinations that have been made, because the reports are made on the quantity of Usneae lichen.

I.6. Obtaining of the *Usneae lichen* extracts

In order to obtain the extracts of *Usnea barbata* (L.) F.H.Wigg. harvested from the Călimani mountains, Suceava county, *Usneae lichen* was used. In this first study, 3 types of extracts were made, using as solvents: distilled water, absolute ethyl alcohol and acetone. The acetone and ethanolic extracts were obtained by cold maceration, and the aqueous extract was obtained by the method of hot reflux.

Results and discussions

The 3 extracts obtained have different colors and aspects: the aqueous extract is slightly opalescent and has a yellow-brown coloring, the acetone one is clear and yellow-straw color, and the ethanolic one is clear and yellow-brown, similar to the aqueous extract. .

I.7. Identification of active metabolites

Reduced compounds (polyphenols) and polysaccharides were identified in the extracts obtained, in the aqueous extract by the following reactions: reaction with the Fehling reagent, with the formation of the red-brick precipitate; reaction with Folin Ciocâlteu reagent, with the formation of blue coloration; reaction with the Tollens reagent, with the formation of the silver mirror; precipitation reaction with methanol, with the formation of the white flocon precipitate.

I.8. Determination of the polyphenols and usnic acid content of the *Usnea barbata* (L.) F.H. Wigg. extracts and evaluation of their antioxidant action

In the present study, in order to determine the basic constituents of *Usnea barbata* (L.) F.H.Wigg., the extracts were prepared in different solvents: water, acetone and 96% ethanol. The identification and determination of the content in usnic acid and polyphenols was performed by HPLC method; the determination of polyphenols was also carried out by a colorimetric method, according to European Pharmacopoeia 9.0.

I.8.1. Determination of the total polyphenols content

For this purpose, a colorimetric method, mentioned in European Pharmacopoeia 9.0 [234], was used.

Principle of the method: The method is based on determining the intensity of the blue staining of the molybdenum oxides formed by reducing the polyphenols of the Folin-Ciocalteu reagent (phosphomolibdolframic acid).

Results and discussions

Since this determination was made in four extracts obtained in different solvents, water, 40% ethanol, 96% ethanol, acetone, the obtained results were synthesized and compared comparatively, thereafter. Analyzing the presented data, it was observed that in the ethanol extract 40% is found the highest content of total polyphenols, followed at a very small distance from the value calculated for the ethanol extract 96%. In the aqueous extract and in the acetonic macerate, the values of the total polyphenols were close, but lower than in the ethanolic extracts.

I.8.2. Analysis of the polyphenols from *Usneae lichen* extract by the high performance liquid chromatography method

For the separation, identification and quantification of phenolic compounds, a standardized HPLC method for the determination of the total polyphenols, as described by USP 30-NF25 monograph [236], was adapted. 11 standard polyphenols were used as reference substances.

Results and discussions

It is observed that in the studied extracts of *Usneae lichen*, only a part of the 11 polyphenols used as reference substances was identified. The mentioned polyphenols were found only in ethanolic and aqueous extracts, and some of them were common to both *Usneae lichen* extracts. None of the 11 standard polyphenols was found in the acetone macerate.

I.8.3. Determination of the usnic acid content

The usnic acid is one of the most widespread secondary metabolites of lichens; it has dibenzofurane structure and it can be isolated from most species of the genus *Usnea* Dill.ex Adans., having multiple known biological actions [144, 150, 155, 161, 162, 165].

Material and method

For the separation, identification and quantification of the usnic acid, a standardized HPLC method has been adapted for the determination of the total polyphenols, as described in the literature; the reference substance was the usnic acid.

Results and discussions

It has been observed that the highest content of the usnic acid is found in the acetone extract followed by the ethanolic extract. The aqueous extract from *Usneae lichen* contains the smallest amount of usnic acid.

I.8.4. Evaluation of the antioxidant capacity of the *Usneae lichen* extracts by DPPH method

Material and method

The antioxidant capacity was spectrophotometrically determined by the DPPH radical scavenging method, a standardized method for evaluating the potential of the phenolic compounds to inactivate the free radicals. This method is achieved by measuring the neutralization capacity of DPPH (diphenylpicrylhydrazyl) radicals by the analyzed solutions; the purple color of the initial DPPH solution

at 517 nm is changed to light yellow, as it appears in reduced form after the contact between the DPPH solution and the polyphenols [89-105, 325].

Results and discussions

It has been observed that the ethanolic extract has the highest antioxidant activity, followed by the aqueous extract and the acetone extractAt the highest concentration, *Usneae lichen* ethanolic extract has a DPPH free radical capture activity greater than 50%, thus it was possible the establishing IC 50 for this extract.

I.9. Obtaining the *Usneae lichen* dry acetone extract and analyzing it by an ultra- high performance liquid chromatography method

I.9.1. Obtaining the *Usneae lichen* dry acetone extract

Material and method

In order to obtain the dry extract, *Usneae lichen* was ground in powder form and kept with acetone, in a continuous reflux on Soxhlet. After the completion reflux , the solvent evaporation was performed and the dry acetone extract was transferred to a glass vessel with a sealed lid and kept in the freezer, at a temperature below -20 ° C, until the further processing [120].

Results and discussions

The dry extract obtained has a pasty consistency, green-brown coloration, strong acetonic odor and quick taste.

I.9.2. Ultra High Performance Liquid Chromatography (UHPLC)

Material and method

The identification and determination of the content of usnic acid was performed by the UHPLC method; analysis sample: dried extract of *Usneae lichen*, solubilized in DMSO. Solubilization in DMSO was performed because acetone is not a suitable solvent for UHPLC analysis (it absorbs strongly and deforms the peak of the solution to be analyzed); also, for cell culture studies, DMSO, unlike acetone, is not cell toxic [263]; reference substance: usnic acid in DMSO.

Results and discussions

The content of usnic acid determined in the dry acetonic extract is significantly higher than in the acetic acid macerate. In the chromatograms of the diluted solutions, the presence of other organic compounds, still unidentified, that potentiate the therapeutic effect of the dry extract was observed.

II. Research on the cytotoxic properties of the *Usnea barbata* (L.) F.H.Wigg. dry extract on *Artemia salina* L.

Hypothesis of this study: The chemical constituents in the *Usneae lichen* dry extract have cytotoxic action, which could cause the death of larvae of *Artemia salina* L.

Purpose of the study: preliminary evaluation of the cytotoxic potential of the *Usneae lichen* dry extract on *Artemia salina* L.

II.1. Material and method

This preliminary cytotoxicity study was performed on larvae of *Artemia salina* L., which were contacted with dilute solutions of the dry extract of *Usneae lichen* (EUB). Macroscopic effects were quantified by measuring the survival level of the larvae of *Artemia salina* L., under the conditions of exposure to different concentrations of dried extract of *Usneae lichen* in DMSO for a limited time.

II.2. Results and discussions

After the contact with EUB DL50 was calculated; the obtained results showing that the dry extract of *Usneae lichen* has an obvious cytotoxic action, directly proportional to the dose and the contact time.

III. Evaluation of the anti-tumor action of the *Usneae lichen* dry acetone extract on lingual squamous cell carcinoma - CAL 27 (ATCC® CRL-2095 ™)

Hypothesis of this study: The *Usneae lichen* dry extract (EUB) has antitumor action on lingual squamous cell carcinoma.

Purpose of the study: to evaluate the antitumor action according to the concentration of the extract and the exposure time and to determine the possible mechanisms by which the death of CAL 27 tumor cells occurs.

III.1. Preparation of the used material

Cells of human tumor line CAL 27 (ATCC® CRL-2095 ™) represented by squamous epithelial cells isolated from lingual carcinoma were counted, while assessing cell viability.

III.2. Determination of the morphological changes

This determination was made by applying diluted solutions of the dried extract of *Usneae lichen* (EUB) on the cultures of tumor cells CAL 27. Morphological changes of CAL 27 cells after contact with the tested solutions were detected under microscope.

Results and discussions

The analysis of the cell cultures under microscope, after their contact with EUB, showed that the morphology of the CAL 27 cells changed differently, depending on the age of the cell culture, the exposure time and the concentration of the tested EUB solution. Previous observations have led to the conclusion that the morphological changes were due exclusively to the dried extract of *Usneae lichen*.

III.3. Total protein dosage

Given that proteins are fundamental to maintaining life, it is estimated that decreasing the amount of total protein leads to cell death. Based on these considerations, the working hypothesis of this subchapter is that the application of different concentrations of EUB generates the death of CAL 27 tumor cells by this mechanism.

Principle of the method: Coomassie Brilliant Blue G (CBBG) dye, in contact with proteins, generates a blue complex; At the same time as the formation of the blue complex, there is also a change in the maximum absorption capacity.

Results and discussions

Analyzing the obtained results it is observed that the amount of soluble proteins in the CAL 27 tumor cells is directly proportional to the concentration of EUB.

III.4. Determination of the cell viability by MTT method

Principle of the method: The colorimetric method with 3- (4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium (MTT) bromide is based on the ability of mitochondrial dehydrogenases from living cells to convert the yellow water-soluble substrate (MTT) to formazan, color dark blue, insoluble in water; the amount of formazan produced is directly proportional to the number of living cells [315, 316, 318].

Results and discussions

The most intense cytotoxic activity was manifested at the 24-hour contact of CAL 27 cells with EUB.

III.5. Evaluation of the cell apoptosis by flow-cytometry

Principle of the method: Fluorochrome-labeled annexin V is used to detect phosphatidyl serine exposed to the surface of the apoptotic cell membrane by flow cytometry. The conjugation between Annexin V and fluorescein (FITC - Fluorescein isothiocyanate) facilitates rapid quantification of fluorescently labeled apoptotic cells. The processing of cells for evaluation of results is performed using the kit "eBioscience ™ Annexin V-FITC Apoptosis Detection [344].

Results and discussions

The analysis of the obtained data shows that the *Usneae lichen* dry acetone extract induces apoptosis of the CAL 27 tumor cells directly proportional to the concentration of the solution used and the exposure time.

III.6. Evaluation of the action of the *Usneae lichen* dry acetone extract on the activity of antioxidant enzymes in the tumor cells CAL 27

Hypothesis of this study: The dry acetonic extract of *Usneae lichen* has the action of stimulating the antioxidant enzymes of CAL 27 cells.

Purpose of the study: to determine the activity of the main antioxidant enzymes in CAL 27 cells: SOD, CAT and POD and to evaluate their stimulation level by the dried extract of *Usneae lichen*, in the context of the cytotoxic effect exerted by EUB on the tumor cells and in correlation with the concentration of the extract and with contact time.

III.6.1. Determination of the superoxide dismutase activity

III.6.2. Spectrophotometric determination of the catalase activity

III.6.3. Determination of the peroxidase activity

Results and discussions

There is a slight stimulation of the SOD activity by EUB, as well as a strong stimulation of the CAT and POD activities.

The effect of EUB on antioxidant enzymes, closely related to its cytotoxicity on CAL 27 cells and its influence on the total protein concentration in CAL 27 cells, was analyzed in the same study (subchapters III.4. And III.5.).

IV. Determination of the antibacterial effects of the *Usnea barbata* (L.) F.H. Wigg extracts on the bacterial species isolated from oropharynx

Hypothesis of this study: the existence of possible antibacterial effects of the *Usneae lichen* extracts in ethanol, acetone and water.

The aim of the study: to evaluate the intensity of the inhibitory effect on the bacterial species isolated from the oro-pharynx, for each type of *Usneae lichen* extract, separately.

Results and discussion

Comparing the antibacterial action of the acetonic and ethanolic extracts of *Usneae lichen* on both genera of Gram-positive bacteria tested, it was observed that the zones of inhibition are very close in size in the case of *Staphylococcus aureus* and *Streptococcus oralis*, while in *Staphylococcus epidermidis* they are considerably larger. It can be stated, by analyzing the results, that the aqueous extracts are practically devoid of antibacterial activity.

Of the Gram-negative bacteria, *Escherichia coli* is partially sensitive, as resistant mutant strains develop in existing areas of inhibition. However, *Pseudomonas aeruginosa*, known for its resistance to antibacterial agents, has high levels of sensitivity compared to *Escherichia coli.*

FINAL CONCLUSIONS

1. **Phytochemical research carried out on *Usnea barbata* (L) F.H.Wigg showed: mucilages, polyphenols and other reducing compounds, as well as usnic acid.**

2. **The highest polyphenols content is found in ethanol extracts; In the aqueous and acetonic extracts, the calculated total polyphenol content values were very close, being lower than in the ethanol extracts.**

3. **The results of the HPLC determination showed that the acetonic extract obtained by maceration had the highest content of mild acid, followed - in descending order - by the ethanolic and aqueous extract.**

4. **The *Usneae lichen* acetone, ethanolic and aqueous extracts have an antioxidant action directly proportional to the polyphenols content; the ethanolic extract has the most intense antioxidant activity.**

5. **The *Usneae lichen* dry acetone extract has an usnic acid content by 8 times higher than the acetone macerate.**

6. ***Usnea barbata* (L.) F.H.Wigg. dry acetone extract presents an obvious cytotoxic action, quantified by the high percentage of mortality of larvae from *Artemia salina* L. directly proportional to the dose and the contact time.**

7. ***Usnea barbat*a (L.) F.H.Wigg. dry acetone extract has a cytotoxic effect on the tumor cell line CAL27, directly proportional to the concentration of the used solutions.**

8. **The cytotoxicity of EUB can also be explained by the decrease of the total proteins, depending on the concentration of the extract;**

9. **The EUB-induced cytotoxicity is higher after 24 hours, compared with that recorded after 48 hours - slightly lower; this small difference is probably due to the intracellular repair process.**

10. **Induction of the apoptosis is directly proportional to the EUB concentration and the contact time.**

11. **EUB stimulates the activity of the main antioxidant enzymes:**

The SOD activity is stimulated slightly, while the CAT and POD activities increase significantly;

The significant increase of the CAT and POD activity compared to SOD could be justified by the increase of the production of H2O2 (the substrate on which both enzymes act) caused by the EUB triggering the process of formation of SRO in mitochondria;

From an oxidative-reducing point of view, EUB acts pro-oxidant on the tumor cells, causing an over-production of SRO; by inducing a strong oxidative stress in tumor cells, EUB decreases the cell viability, resulting in apoptosis.

12. **The *Usneae lichen* ethanolic and acetone extracts have antibacterial effect:**

***Staphylococcus aureus* has been shown to be sensitive to the action of the *Usneae lichen* ethanolic and acetone extracts.**

***Pseudomonas aeruginosa* shows a significant sensitivity to the action of the *Usneae lichen* acetone and ethanolic extracts.**

13. **The results of this study demonstrate that the *Usnea barbata* (L.) F.H.Wigg. extracts could be used effectively in the oral infections in which bacteria resistant to common antibacterial therapy are involved, thus completing the conventional treatment with antibiotics.**

REFERENCES

1. Prateeksha, G., Paliya, B.S., Bajpai, R.. et al., The genus *Usnea*: A potent phytomedicine with multifarious ethnobotany, phytochemistry and pharmacology, RSC Adv., 2016; 6: 21672 –21696
2. *** Index Fungorum, *Usnea barbata* (L.)F.H.Wigg., www.indexfungorum.org /names/ NamesRecord.asp?RecordID=408326
3. Devisha, D., Sarkar, S., Recent advancement in periodontics, I.J.S.N., 2019, 10 (1): 1-4.
4. *** WHO, Noncommunicable diseases country profiles 2018, https://www.who.int/nmh/publications/ncd-profiles-2018/en/ Publication date: September 2018 .
5. Ozturk,S., Erkisa, M., Oran,S., Ulukaya, E., Celikler, S. & Ari, F., Lichens exerts an anti-proliferative effect on human breast and lung cancer cells through induction of apoptosis, Drug and Chemical Toxicology, 2019, DOI: 10.1080/01480545.2019.1573825
6. Kriebel K, Hieke C, Müller-Hilke B, Nakata M., Kreikemeyer B, Oral Biofilms from Symbiotic to Pathogenic Interactions and Associated Disease - Connection of Periodontitis and Rheumatic Arthritis by Peptidylarginine Deiminase, Front. Microbiol.,2018; 9(53):1-14
7. Monea A.,Monea, M.,et al., Tongue microflora and periodontal disease, European Scientific Journal , 2014; 10(36):12-17
8. ***National Cancer Institute, „What is cancer", www.cancer.gov/about-cancer/understanding/ #related-diseases. Accesat, iunie 2019
9. ***National Cancer Institute, „What you need to know about oral cancer." www.cancer.gov/cancertopics/wyntk/oral. Accesat, noiembrie 2018.
10. Jain, S., Dwivedi, J., Jain, PK., Satpathy, S., Patra, A., Medicinal Plants for Treatment of Cancer: A Brief Review, Pharmacognosy Journal, 2016; 8(2):87-102.
11. Gontier, N., Symbiosis, History of. In: Kliman, R.M. (ed.), Encyclopedia of Evolutionary Biology, 2016; 4: 272–281. Oxford: Academic Press
12. Galinato MGM, Baguinon JRC, Santiago KAA –Review of the lichen genus Usnea in the Philippines. Studies in Fungi, 2018; 3(1): 39–48
13. ***CNALH. 2019. http//:lichenportal.org/portal/index.php. Accessed on June 09.
14. Boustie, J., Grube, M., Lichens as a promising source of bioactive secondary metabolites, PlantGenetic Resources 2005; 3: 273-287.
15. Pereira EC, Martins MCB, de Lourdes L., Buril M., Santiago R., et al. , Biologically-Active Compounds from Brazilian Lichens and their Affinity with Ether. J Drug Des Res.,2017; 4(6): 1057
16. Nguyen T., Chollet-Krugler, M., Sauvager, A., Boustie J., Chemical constituents of the lichen *Dermatocarpon luridum* (With.) J.R.Laundon.. Journées internationales AFERP & STOLON, May 2013, Bruxelles, Belgium. ⟨hal-00873172⟩
17. Ohmura,Y., A Synopsis of the Lichen Genus *Usnea* (Parmeliaceae, Ascomycota) in Taiwan, Mem. Natl. Mus. Nat. Sci., 2012; 48: 91-137
18. Aschenbrenner, I.A., Cernava, T., Berg, G., Grube, M., Understanding Microbial Multi-Species Symbioses, Front Microbiol. 2016; 7: 180.
19. Radlane, T., Torra, T., et al., Key to European species, in The Diversity of Lichenology: Jubilee

Volume, A. Thell, M. R. D. Seaward & T. Feuerer (eds). Bibliotheca Lichenologica, 2009; 100: 419–462, J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Berlin · Stuttgart

20. ***Association Française de Lichénologie, www.afl-lichenologie.fr/ PhotosAFL/Photos_AFL_*Usnea_barbata*.htm
21. Brezeanu, A., Voicu, D., Ultrastructural peculiarities of the *Usnea barbata* (L.) Mott., Mycobiont and phycobiont cells in "in vivo" and "in vitro" culture, Rom J. Biol. – Plant. Biol., 2008; 53(2): 63-69
22. Hibbett, D.S.; et al., A higher level phylogenetic classification of the Fungi, Mycological Research, 2007; 111 (5): 509–547.
23. Lücking, R., Hodkinson B., Leavitt, SD., The 2016 classification of lichenized fungi in the *Ascomycota* and *Basidiomycota* – Approaching one thousand genera: The Bryologist 2017; 119(4): 361-416.
24. Truong, C., Rodriguez JM, and Clerc, P., Pendulous *Usnea* species (*Parmeliaceae*, lichenized *Ascomycota*) in tropical South America and the Galapagos, The Lichenologist 2013; 45(4): 505–543
25. Stănescu, U., Hancianu, M., Cioanca,O., Plante medicinale de la A la Z, Editura Polirom 2014; 1261-1265
26. *** *Usnea barbata,* Encyclopedia of Life. Available from https://eol.org/pages/11494366/names
27. Ranković, B., Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential, Edition: Springer International Publishing, 2015; ISBN: 978-3-319-13374-4
28. Bucur, A., Adenopatia metastatică loco-regională după tumori maligne oro-maxilo- -faciale, part III, Medicina Stomatologică, 2005; 9 (6)
29. Newman, D., Cragg, GM, Natural products as sources of new drugs over the 30 years from 1981 to 2010, J. Nat. Prod., 2012; 23 : 311-335
30. Oprică L., Metaboliți secundari din plante, origine, structură, funcții, Editura Universității Alexandru Ioan Cuza Iași, 2016; ISBN: 978-606-714-253-2
31. Kabera, J., Semana, E., Mussa, A., et al Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties, Journal of Pharmacy and Pharmacology 2014; 2: 377-392
32. Salgado, F., Albornoz, L., Cortéz, C., Stashenko, E. et al., Secondary Metabolite Profiling of Species of the Genus *Usnea* by UHPLC-ESI-OT-MS-MS Molecules, 2018 ; 23(1): 54.
33. Boustie J., Tomasi,S., Grube, M., Bioactive lichen metabolites: alpine habitats as an untapped source, Phytochemistry Reviews, Springer Verlag, 2011; 10 (3): 287-307.
34. Bhattacharyya S et al., Lichen Secondary Metabolites and Its Biological Activity. American Journal of PharmTech Research, 2016; 28-44
35. Vanaja et al., Biology of Lichen polysaccharides and its Applications-a Review, Proceedings of NCIB, 2017; 64-72
36. Mitrović, T., Stamenković, S., Cvetković, V.. et al., Lichens as source of versatile bioactive compounds, Biologica Nyssana, 2011; 2(1): 1-6
37. Shukla, P., Upreti, D.K.,Tewari, L.M., Secondary metabolite variability in lichen genus *Usnea* in India: A potential source for bioprospection, G- Journal of Environmental Science and Technology, 2014; 2(3): 29-40
38. ***National Cancer Institute, Oxidative methabolism, definition, https://www. cancer.gov/publications/dictionaries/cancer-terms/def/oxidative-metabolism, accesed 05.08.2019
39. Cansaran, D. et al., Determination of Usnic Acid Content in Some Lichen Species Found in Anatolia,

Journal of Applied Biological Sciences 2008; 2 (3): 41-44

40. Cansaran, D. et al., Identification and Quantitation of Usnic Acid from the Lichen *Usnea* Species of Anatolia and Antimicrobial Activity, Z. Naturforsch., 2006; 61(c): 773-776
41. Shrestha, G., St. Clair, L., O'Neill, L., The immunostimulating role of lichen polysaccharides: a review – abstract, Phytother. Res. 2015; 29(3): 317-322
42. Olafsdottir ES, Ingolfdottir K., Polysaccharides from lichens: structural characteristics and biological activity, Planta Med 2001; 67: 199–208
43. Olafsdottir ES, Omarsdottir S, Smestad Paulsen B et al ,Rhamnopyranogalactofuranan, a new immunologically active polysaccharide from *Thamnolia subuliformis*. Phytomedicine, 1999; 6: 273–279
44. Olafsdottir ES, Omarsdottir S, Paulsen BS et al ,Immunologically active O6-branched (1–3)-b-glucan from the lichen Thamnolia vermicularis var. subuliformis, Phytomedicine 2003; 10: 318–324
45. Podterob AP, Chemical composition of lichens and their medical application. Pharm Chem J., 2008; 42: 582–588
46. Muggia L, Grube M., Fungal Diversity in Lichens: From Extremotolerance to Interactions with *Algae*, Life (Basel), 2018; 8(2): 15-19
47. Vicol, I., Effect of old-growth forest attributes on lichen species abundances: a study performed within Ceahlău Naţional Park (România) – abstract - Cryptogamie, Mycologie, 2015; 36 (4): 399-407
48. Marmor, L., Torra, T., et. al., Lichens on *Picea abies* and *Pinus sylvestris* – from tree bottom to the top, The Lichenologist, 2013, 45(1): 51–63
49. Francolini, P. Norris, A. Piozzi, G. Donelli, and P. Stoodley, Usnic Acid, a Natural Antimicrobial Agent Able To Inhibit Bacterial Biofilm Formation on Polymer Surfaces, Antimicrobial Agents and Chemotherapy, 2004; 4360–4365
50. Maciag-Dorszynska, M., Wezgrzyn, G., Guzow-Krzeminska, B., Antibacterial activity of lichen secondary metabolite usnic acid is primarily caused by inhibition of RNA and DNA synthesis, FEMS Microbiol Lett, 2014; 353: 57–62
51. Gupta, K.& Verma S& S. Gupta S.& A. Singh & A. Pal & S. K. Srivastava & P. K. Srivastava & S. C. Singh & M. P. Darokar, Membrane-damaging potential of natural L-(−)-usnic acid in *Staphylococcus aureus*, Eur J Clin Microbiol Infect Dis., 2012; 31:3375–3383
52. Madamombe I.T., Afolayan A.J., Evaluation of antimicrobial activity of Extracts from South Africa *Usnea barbata*, Pharm.Biol. 2003; 41(3): 199-202
53. Srivastava P, Upreti DK, Dhole TN, Srivastava AK, Nayak MT., Antimicrobial Property of Extracts of Indian Lichen against Human Pathogenic Bacteria, Interdiscip Perspect Infect Dis., 2013: 709348
54. Tozatti MG, Ferreira DS, Flauzino LG, Moraes Tda S, Martins CH, Groppo M, Andrade e Silva ML, Januário AH, Pauletti PM, Cunhaa WR., Activity of the Lichen *Usnea steineri* and its Major Metabolites against Gram-positive, Multidrug-resistant Bacteria., Nat Prod Commun. 2016; 11(4):493-496.
55. Manoharachary, C., Nagaraju, D., Antimicrobial and Antifungal activity of *Leptogium Javanicum* Mont. and *Usnea ghattensis* Awasthi C., International Journal of Modern Chemistry and Applied Science, 2016; 3(3): 444-445
56. Çobanoğlu, G. et.al., Evaluation Of Antimicrobial Activity Of The Lichens *Physcia Aipolia, Xanthoria*

Parietina, Usnea Florida, Usnea Subfloridana And *Melanohalea Exasperata*, Modern Phytomorphology, 2016; 10: 19–24

57. Matvieieva, N.A., Pasichnyk, L.A., Zhytkevych, N.V., Antimicrobial activity of extracts from Ecuadorian lichens, Mikrobiolohichnyĭ zhurnal, 2015; 77(3): 23-27
58. Kamal, S., Manish, S., Savita, J. et.al., Assessment of Antibacterial Activity of *Usnea* Species of Shimla Hills, Int.J.Curr. Microbiol.App. Sci., 2015; 4(7): 413-425
59. Gajendra S, Clair LLS. Lichens: a promising source of antibiotic and anticancer drug, Phytochemistry Reviews, 2013; 12: 229–244
60. Behera BC, Verma N, Sonone A, Makhija U., Antioxidant and antibacterial activities of lichen *Usnea ghattensis* in vitro, Biotechnology Letters, 2005; 27: 991– 995
61. Idamokoro, E., Masika, P., Muchenje, V., 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 2014; 57(25): 1-9
62. Elo H, Matikainen J, Pelttari E., Potent activity of the lichen antibiotic (+)-usnic acid against clinical isolates of vancomycin resistant enterococci and methicillin-resistant *Staphylococcus aureus*, Naturwissenschaften 2007; 94: 465–468
63. Kosanić, M., Ranković, B., Screening of antimicrobial activity of some lichen species in vitro, Kragujevac J. Sci., 2010; 32: 65-72
64. Lauterwein, M., Oethinger, M.,Belsner, K. et al., In Vitro Activities of the Lichen Secondary Metabolites Vulpinic Acid, (1)-Usnic Acid, and (2)-Usnic Acid against Aerobic and Anaerobic Microorganisms, Antimicrobial Agents and Chemotherapy, 1995; 2541–2543
65. Lei Guo et. al., Review of Usnic Acid and *Usnea barbata* Toxicity, Journal of Environmental Science and Health, 2008; Part C, 26:317–338.
66. Lucarini, L., Tozatti, M., Antimycobacterial activity of *Usnea steineri* and its major constituent (+)-usnic acid, African Journal of Biotechnology, 2012; 11(20): 4636-4639
67. Oscar E., Rodríguez, A., William, A., Andrade, B., Antimicrobial activity of lichens in the upper basin of Bogotá river, NOVA., 2015; 13 (23): 65-72
68. Ullah, M., et al., Bacterial neuraminidase inhibition by phenolic compounds from *Usnea longissima,* South African Journal of Botany, 2018;
69. Perry, N., Benn, M., et. al., Antimicrobial, Antiviral and Cytotoxic Activity of New Zealand Lichens, The Lichenologist , 1999 ; 31(6): 627–636
70. Prabhu S.S., Sudha S.S., Evaluation of the antibacterial properties of some Lichen species against human pathogens, Int. J. Adv. Res. Biol. Sci.,2015; 2(4):177-181
71. Santiago, K., Sangvichien, E., Boonpragob, K., Secondary metabolic profiling and antibacterial activities of different species of *Usnea* collected in Northern Philippines, Mycosphere, 2013; 4(2): 267–280
72. Thippeswamy, B., Naveenkumar, K. et. al., Antimicrobial activity of ethanolic extract of *Usnea longissima*, Journal of Experimental Sciences, 2011; 2(12): 01-03
73. Santiago, K., Borricano, J. et al., Antibacterial activities of fruticose lichens collected from selected sites in Luzon Island, Philippines, Philippine Science Letters 2010; 3(2), 18–28
74. Shanmugam, P., et al., Evaluation of Antimicrobial, antioxidant and anticancer activities of few

macrolichens collected from Eastern Ghats of Tamil Nadu, India, Int. Res. J. Pharm. 2017; 8 (3): 39-43

75. Paudel, B., Bhattarai, K., Bhattarai, H.D., Antimicrobial and antioxidant activities of two polyketides from lichen-endophytic fungus Preussia sp., Z. Naturforsch., 2018; C 73(3-4)
76. Pathak, A., Upreti, D.K., Dikshit A., Antidermatophytic Activity of the Fruticose Lichen *Usnea orientalis*, Medicines 2016; 3(24): 2-6
77. Schmeda-Hirschman, G., Tapia, A., Lima, B. et al., A new antifungal and antiprotozoal depside from the Andean lichen *Protousnea poeppigii*. Phytotherapy Research, 2008; 22(3): 349–355
78. Pires, R.H., Lucarini, R., Effect of Usnic Acid on *Candida orthopsilosis* and *C. Parapsilosis*, Antimicrob. Agents Chemother., 2012; 56(1): 595.
79. Rusu, E., Cristescu, C., Dimorphism regulation through quorum sensing molecules in *Candida albicans* species, Revista Română de Boli Infecţioase 2012, 15(2)
80. Rukayadi. Y., Diantini. A., Lestari K., Anti Fungal Activity of Methanolic Extract of *Usnea* sp. against *Malassezia furfur*, Bionatura-Jurnal Ilmu-ilmu Hayati dan Fisik , 2012; 14 (1): 31 - 37
81. Vijayan, P., Raghu, C. , Ashok, G. , Dhanaraj S.A. & Suresh, B. , Antiviral activity of medicinal plants of Nilgiris, Indian J Med Res., 2004; 120: 24-29
82. Scirpa P, Scambia G, Masciullo V, et.al., A zinc sulfate and usnic acid preparation used as post-surgical adjuvant therapy in genital lesions by *Human Papillomavirus*, Minerva Ginecol, 1999; 51:255–260.
83. Yamamoto, Y, Miura Y, Kinoshita Y, et. al., Screening of tissue cultures and thalli of lichens and some of their active constituents for inhibition of tumor promoter-induced Epstein-Barr virus activation, Chem Pharm Bull (Tokyo) 1995; 43: 1388–1390.
84. Fournet, A. et. al., Activity of compounds isolated from Chilean lichens against experimental cutaneous leishmaniasis, Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 1997; 116: 51–54
85. Hobbs, C. , Usnea: The Herbal Antibiotic. Capitola, CA: Botanica Press, 1986
86. Stephen Harrod Buchner: Herbal Antibiotics; e-book version 2.1 – 2016: 208 – 219
87. Molnár K, Farkas E., Current results on biological activities of lichen secondary metabolites: a review. Z. Naturforsch, 2010; C65:157–173
88. Pavithra G.M., Vinayaka K.S., Rakesh K.N., et al., Antimicrobial and antioxidant activities of a macrolichen *Usnea pictoides* G. Awasthi (*Parmeliaceae)*, Journal of Applied Pharmaceutical Science, 2013; 3 (08) :154-160.
89. Panieri, E., and M.M. Santoro. ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell Death Dis. 2016; 7:e2253. https://doi.org/101038/cddis.2016.10
90. Vucˇetic′ M, Cormerais Y, Parks SK and Pouysségur J, The Central Role of Amino Acids in Cancer Redox Homeostasis: Vulnerability Points of the Cancer Redox Code. Front. Oncol. 2017; 7:319
91. Asaduzzaman K.,Mousumi T.,Dian-zheng Z., Han-chun C., Antioxidant Enzymes and Cancer, Chin J Cancer Res, 2010; 22(2):87-92
92. Odabasoglu, F, Aslan, A, Cakir A, Suleyman H, Karagoz Y, Halici M and Bayir Y. Comparison of antioxidant activity and phenolic content of three lichen species, Phytother. Res. 2004; 18: 938-941.
93. Odabasoglu, F, Aslan, A, Cakir A, Suleyman H, Karagoz Y,Bayir Y and Halici M., Antioxidant activity,

reducing power and total phenolic content of some lichen species, Fitoterapia, 2005; 76: 216-219

94. Behera BC, Verma N, Sonone A., Makhija U., Determination of antioxidative potential of lichen *Usnea ghattensis* in-vitro, LWT-Food Sci. Technol., 2006; 39: 80-85.
95. Schumacker, P.T. Reactive oxygen species in cancer cells: live by the sword, die by the sword. Cancer Cell., 2006; 10:175–176. https://doi.org/10.1016/j.ccr.2006.08.015
96. Kosanić M, Ranković B and Vukojević J. Antioxidant properties of some lichen species. J. Food Sci. Technol.,2011; 48: 584-590.
97. Miriyala, S., I. Spasojevic, A. Tovmasyan, D. Salvemini, Z. Vujaskovic, D. St.Clair, and I. Batinic-Haberle. 2012. Manganese superoxide dismutase, MnSOD and its mimics. Biochim. Biophys. Acta. 1822:794–814. https://doi.org/10.1016/j.bbadis.2011.12.002
98. *** Nebraska Redox Biology Center Educational Portal http://genomics.unl.edu/RBC_EDU/hp.html
99. Hempel, N., P.M. Carrico, and J.A. Melendez, Manganese superoxide dismutase (Sod2) and redox-control of signaling events that drive metastasis. Anticancer Agents Med. Chem. 2011;11:191–201. https://doi.org/10.2174/ 187152011795255911
100. Verma, P., Paswan S.K., Abhisek Raj, A. et al., Hematological, antioxidant and protective performance of *Usnea longissima* on chemical induced hepatotoxicity in experimental animals, Journal of Coastal Life Medicine 2017; 5(5): 224-232
101. Susithra, E., et al. , Evaluation of In-vitro Antioxidant activity of isolated compounds of lichen *Usnea undulata* , Journal of Pharmacy Research 2011; 4(2): 352-355
102. Jie Wang & Jing Yi, Cancer cell killing via ROS: To increase or decrease, that is the question, Cancer Biology & Therapy, 2008; 7 (12):1875-1884, DOI: 10.4161/ cbt.7.12.7067
103. Hurt, E.M., S.B. Thomas, B. Peng, and W.L. Farrar. Molecular consequences of SOD2 expression in epigenetically silenced pancreatic carcinoma cell lines. Br. J. Cancer. 2007; 97:1116–1123. doi:10.1038/sj.bjc.6604000
104. Aurer, Modern understanding of periodontal diseases, Rad 514 Medical Sciences, 2012; 38 : 49-59
105. Dhar, S.K., and D.K. St Clair. 2012. Manganese superoxide dismutase regulation and cancer. Free Radic. Biol. Med., 2012; 52:2209–2222.
106. Kohlhardt-Floehr C, Boehm F, Troppens S, Lademann J, Truscott TG., Prooxidant and antioxidant behaviour of usnic acid from lichens under UVB-light irradiation - Studies on human cells. J Photochem Photobiol B 2010; 101: 97–102.
107. Müller K., Pharmaceutically relevant metabolites from lichens. Applied Microbiology and Biotechnology , 2002; 56(1/2): 9-16.
108. Sheiham, A., Oral health, general health and quality of life, Bulletin of the World Health Organization, 2005; 83(9): 641-720
109. Cragg GM, Newman DJ. Nature: a vital source of leads for anticancer drug development. Phytochem Rev 2009; 8: 313–331.
110. Vuorelaa P, Leinonenb M, Saikkuc P, Tammelaa P, Rauhad JP, Wennberge T, et al. Natural products in the process of finding new drug candidates. Curr Med Chem 2004;11:1375-89.
111. Mondal S, Bandyopadhyay S, Ghosh MK, Mukhopadhyay S, Roy S, Mandal C.. Natural products: promising resources for cancer drug discovery. Anti-cancer agents in med chem 2012; 12:

49–75.

112. Dhar, S.K., J. Tangpong, L. Chaiswing, T.D. Oberley, and D.K. St Clair., Manganese superoxide dismutase is a p53-regulated gene that switches cancers between early and advanced stages. Cancer Res, 2011; 71:6684–6695.
113. Demain AL, Viashnav P. Natural products for cancer chemotherapy. Microb Biotechnol 2011; 4: 687-99.
114. Cragg GM, Newman DJ., Plants as a source of anti-cancer agents. J Ethnopharmacol 2005; 100:72-9.
115. Ramya, K., Thirunalasundari T., Pharmaceutically relevant metabolites from Lichens of Kodaikanal, India, Int.J.Curr.Microbiol.App.Sci 2014; 3(11) : 779-788
116. Kunjachan S, Rychlik B, Storm G, Kiessling F, Lammers T. Multidrug resistance: Physiological principles and nanomedical solutions. Adv Drug Deliv Rev 2013; 65:1852- 65.
117. Shrestha, G.et al., Anticancer Activities of Selected Species of North American Lichen Extracts, Phytother Res. 2015; 29(1):100-107.
118. Zugic A., Jeremic I., Isakovic A. and all., Evaluation of Anticancer and Antioxidant Activity of a Commercially Available CO2 Supercritical Extract of Old Man's Beard (*Usnea barbata*). Plos One, 1016; 11(1): 1-14
119. Russo A., Piovano M., Lombardo L., Garbarino J., and Cardile V., Lichen metabolites prevent UV light and nitric oxide-mediated plasmid DNA damage and induce apoptosis in human melanoma cells, Life Sci., 2008; 83: 468 – 474.
120. Wang,W., Niu,S.,Luxin Qiao,L., et al, Usnic Acid as Multidrug Resistance (MDR) Reversing Agent against Human Chronic Myelogenous Leukemia K562/ADR Cells via an ROS Dependent Apoptosis, Hindawi BioMed Research International Volume 2019, Article ID 8727935, 7 pages https://doi.org/10.1155/2019/8727935
121. Maulidiyah, A. et al., Cytotoxic activity of (-)-usnic acid compound from lichen *Usnea* sp.on Murine Leukemia P388 Cells, JIFI., 2015; 13(1):
122. Mayer M., O'Neill M. A., Murry K. E., Santos-Magalhães N. S., et al., Usnic acid: a non-genotoxic compound with anti-cancer properties, AntiCancer Drugs, 2005; 16: 805 – 809
123. Mitrović, T., Stamenković, S., Cvetković, V. et al., Antioxidant, Antimicrobial and antiproliferative activities of five Lichen species, Int. J. Mol. Sci., 2011; 12: 5428-5448
124. Connor, K.M., S. Subbaram, K.J. Regan, K.K. Nelson, J.E. Mazurkiewicz, P.J. Bartholomew, A.E. Aplin, Y.T. Tai, J. Aguirre-Ghiso, S.C. Flores, and J.A. Melendez, Mitochondrial H2O2 regulates the angiogenic phenotype via PTEN oxidation. J. Biol. Chem. 2005;280:16916–16924. doi:10.1074/jbc.M410690200
125. Bessadottir M, Egilsson M, Einarsdottir E, Magnusdottir IH, Ogmundsdottir MH, et al. (2012) Proton-Shuttling Lichen Compound Usnic Acid Affects Mitochondrial and Lysosomal Function in Cancer Cells, Plos One, 2012; 7(12): e51296. doi:10.1371/journal.pone.0051296
126. Singh N, Nambiar D, Kale RK, Singh RP., Usnic acid inhibits growth and induces cell cycle arrest and apoptosis in human lung carcinoma A549 cells. Nutr Cancer 2013; 65(1): 36-43.
127. Triggiani D, Ceccarelli D, Tiezzi A, et al., Antiproliferative activity of lichen extracts on murine myeloma cells. Biologia, 2009; 64: 59–62.

128. Shanmugam, P., et al., Evaluation of Antimicrobial, antioxidant and anticancer activities of few macrolichens collected from Eastern Ghats of Tamil Nadu, India, Int. Res. J. Pharm. 2017; 8 (3): 39-43
129. Zuo S, Wang L, Zhang Y, Zhao D, Li Q, Shao D, Fang X., Usnic acid induces apoptosis via an ROS-dependent mitochondrial pathway in human breast cancer cells in vitro and in vivo. RSC Adv, 2015; 5: 153-162
130. Ari F, Celikler S, Oran S, et al., Genotoxic, cytotoxic, and apoptotic effects of *Hypogymnia physodes* (L.) Nyl. on breast cancer cells. Environ Toxicol 2012; DOI: 10.1002/tox.21809.
131. Backorova M, Backor M, Mikes J, Jendzelovsky R, Fedorocko P. 2011. Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid. Toxicol In Vitro 2011; 25: 37–44.
132. Backorova M, Jendzelovsky R, Kello M, Backor M, Mikes J, Fedorocko P., Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. Toxicol In Vitro 2012; 26: 462-468.
133. Bézivin C, Tomasi S, Lohézic-Le Dévéhat F, Boustie J. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines., Phytomedicine, 2003; 10: 499–503.
134. Bezivin C, Tomasi S, Rouaud I, Delcros JG, Boustie J. 2004. Cytotoxic activity of compounds from the lichen *Cladonia convoluta*, Planta Med 2004; 70: 874–877.
135. Brisdelli F, Perilli M, Sellitri D, et al., Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an in vitro study, Phytother Res 2013; 27: 431–437.
136. Ogmundsdottir HM, Zoega GM, Gissurarson SR et al , Anti-proliferative effects of lichen-derived inhibitors of 5-lipoxygenase on malignant cell-lines and mitogenstimulated lymphocytes. J Pharm Pharmacol, 1998; 50: 107–115
137. Kumar S, Muller K., Lichen metabolites. II. Antiproliferative and cytotoxic activity of gyrophoric, usnic and diffractaic acid on human keratinocyte growth. J Nat Prod 1999; 62: 821–823
138. Brisdelli F, Perilli M, Sellitri D, et al. Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an in vitro study. Phytother Res 2013; 27: 431–437.
139. Einarsdóttir E. et al Cellular Mechanisms of the Anticancer Effects of the Lichen Compound Usnic Acid, Planta Med 2010; 76(10): 969-974
140. Kasimogullari SC, Oran S, Ari F, Ulukaya E, Aztopal N, Sarimahmut M, et al. Genotoxic, cytotoxic, and apoptotic effects of crude extract of *Usnea filipendula* Stirt. in vitro. Turk J Biol 2014; 38: 940-947.
141. Ari F, Aztopal N, Oran S, Bozdemir S, Celikler S, Ozturk S, Ulukaya E. *Parmelia sulcata* Taylor and *Usnea filipendula* Stirt induce apoptosis-like cell death and DNA damage in cancer cells., Cell Prolif 2014; 47: 457-464.
142. Yu, X., Guo, Q., Su, G., Yang, A., Hu, Z., Qu, C., Wan, Z., Li, R., Tu, P., Chai, X., Usnic acid derivatives with cytotoxic and antifungal activities from the lichen *Usnea longissima*., Journal of Natural Products, 2016; 79: 1373–1380.
143. Nguyen TT, Yoon S, Yang Y, Lee HB, Oh S, Jeong MH et al., Lichen secondary metabolites in *Flavocetraria cucullata* exhibit anti-cancer effects on human cancer cells through the induction of apoptosis and suppression of tumorigenic potentials. Plos One, 2014; 9: e111575.

144. Kılıç N, Değerli E, Torun V, Altaytaş F, Cansaran-Duman D, Investigation of synergistic effect of tamoxifen and usnic acid on breast cancer cell line. JSM Biology, 2016; 1: 1006.
145. Nagarathna P.K.M., M. Johnson Wesley, P. Sriram Reddy, Reena. K, Review on Genotoxicity, its Molecular Mechanisms and Prevention, Int. J. Pharm. Sci. Rev. Res., 2013; 22(1), 236-243
146. Koparal AT, Ayaz-Tüylü B, Türk H, In vitro cytotoxic activities of (+)-usnic acid and (−)-usnic acid on V79, A549, and human lymphocyte cells and their non-genotoxicity on human lymphocytes. Nat Prod Res, 2006; 20: 1300-1307
147. Leandro,L.F. et al., Assessment of the genotoxicity and antigenotoxicity of (+)-usnic acid in V79 cells and Swissmice by the micronucleus and cometa assay, Mutation Research, 2013; 753: 101–110
148. Ceker S., Orhan F., Emre Kizil, H.,et. al. Genotoxic and antigenotoxic potentials of two Usnea species – Abstract ,Toxicology and Industrial Health, 2013; 31 (11): 990-999
149. Aslan A, Agar G, Alpsoy L et al, Protective role of methanol extracts of two lichens on oxidative and genotoxic damage caused by AFB(1) in human lymphocytes in vitro, Toxicol and Health, 2012; 28(6):505–512
150. Dincsoy, AB, Cansaran-Duman, D, Changes in apoptosis-related gene expression profiles in cancer cell lines exposed to usnic acid lichen secondary metabolite, Turk J Biol., 2017; 41: 484-493
151. Dobrescu,D., Tanasescu, M., Mezdrea, A. et al., Contributions to the complex study of some lichens-*Usnea* genus. Pharmacological studies on Usnea barbata and Usnea hirta species – abstract-Romanian journal of physiology: physiological sciences / [Academia de Științe Medicale], 1993; 30(1-2):101-107
152. Okuyama. E., Umeyama. K., Yamazaki. M., Kinoshita. Y., & Yamamoto, Y, Usnic acid and diffractaic acid as analgesic and antipyretic components of *Usnea diffracta*. Planta Med 1995; 61:113–115.
153. Odabasoglu F, Cakir A, Suleyman H et al., Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats, J Ethnopharmacol 2006; 103: 59–65.
154. Odabasoglu, F., Suleyman, H., Cakir, A., et al., Effects of water extract of *Usnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats, Phytomedicine, 2005; 12: 656–662.
155. Safak, B., Hakki-Ciftci I., Mehmet -Ozdemir, M., In Vitro Anti-Helicobacter pylori Activity of Usnic Acid, Phytother. Res., 2009; 23: 955–957
156. Lee KA, Kim MS., Antiplatelet and antithrombotic activities of methanol extract of *Usnea longissima* - abstract, Phytother Res. 2005; (12):1061-1064.
157. Alahmadi AA, Usnic acid biological activity: history, evaluation and usage.- abstract, Int J Basic Clin Pharmacol. 2017; 586 (6): 2752–2759
158. Araújo AA, de Melo MG, Rabelo TK et al, (2015) Review of the biological properties and toxicity of usnic acid., Nat Prod Res., 2015; 29(23):2167–2180
159. Malhotra, S.,Subban,R., Singh.,A., Lichens- Role in Traditional Medicine and Drug Discovery, The Internet Journal of Alternative Medicine., 2007 ; 5 (2)
160. Weissbuch, BC, Medicinal Lichens: The Final Frontier, JAHG 2014; 12(2): 23-28
161. Luzina A.O & Salakhutdinov ,F.N., Usnic acid and its derivatives for pharmaceutical use: a patent review (2000-2017), Expert Opinion on Therapeutic Patents, 2018;

162. Luzina,A.O & Salakhutdinov,F.N., Biological Activity of Usnic Acid and its Derivatives: Part 2. Effects on Higher Organisms. Molecular and Physicochemical Aspects, Russian Journal of Bioorganic Chemistry, 2016; 42, (3)249–268.
163. White, P., Oliveira, R., Oliveira, AP et.al., Antioxidant Activity and Mechanisms of Action of Natural Compounds Isolated from Lichens: A Systematic Review, Molecules, 2014; 19(9):14496-14527
164. Cabrera, C., Materia Medica, Usnea spp. Eur J Herbal Med, 1996; 2(2):1113.
165. Correche ER, Carrasco M, Escudero ME et al., Study of the cytotoxic and antimicrobial activities of usnic acid and derivatives, Fitoterapia, 1998; 69: 493–501.
166. Bjerke JW, Elvebakk A, Domínguez E, et al. Seasonal trends in usnic acid concentrations of Arctic, alpine and Patagonian populations of the lichen Flavocetraria nivalis. Phytochemistry. 2005; 66(3): 337-344
167. Nimis PL & Martellos,S., 2017: ITALIC - The Information System on Italian Lichens. Version 5.0. University of Trieste, Dept. of Biology, (http://dryades.units.it/italic)
168. Pompilio A, Riviello A, Crocetta V et al. Evaluation of antibacterial and antibiofilm mechanisms by usnic acid against methicillin-resistant *Staphylococcus aureus*. Future Microbiol 2016; 11:1315–1338
169. Kunjachan S, Rychlik B, Storm G, Kiessling F, Lammers T. Multidrug resistance: Physiological principles and nanomedical solutions. Adv Drug Deliv Rev 2013;65:1852- 1865.
170. Mann J. Natural products in cancer chemotherapy: past, present, and future. Nat Rev Cancer 2002;2(2):143-148.
171. Prakash O, Kumar A, Kumar P, Ajeet. Anticancer Potential of Plants and Natural Products-A Review. Am J Pharmacol Sci 2013;1:104-15.
172. Zambare VP, Christopher LP. Biopharmaceutical potential of lichens. Pharm Biol 2012;50:778-98.
173. Gabriele Pizzino et al., Oxidative Stress: Harms and Benefits for Human Health, Oxidative Medicine and Cellular Longevity, 2017, Article ID 8416763, 13 pages; https://doi.org/10.1155/2017/8416763
174. Klaunig, JE, Wang,Z., Oxidative stress in carcinogenesis, Current Opinion in Toxicology, 2018; 7 :116-121
175. Liou G.I., Storz P., Reactive oxygen species in cancer, Free Radic Res., 2010; 44(5): 1-31.
176. Jasen Kundalić et al., Oxidative stress in the pathogenesis of periodontal disease, Acta Medica Medianae 2016, Vol.55(4): 66-71
177. Wang et al., Oxidative Stress and Antioxidant System in Periodontitis, Frontiers in Physiology , 2017 ; 8: Article 910, doi: 10.3389/fphys.2017.00910
178. ***WHO, Oral Health, 2018, https://www.who.int/news-room/fact-sheets/detail/oral-health
179. Lubna Tahir and Rabia Nazir, Dental Caries, Etiology, and Remedy through Natural Resources, Dental Caries - Diagnosis, Prevention and Management, Zühre Akarslan, IntechOpen, 2018: DOI: 10.5772/intechopen.75937. Available from: https://www.intechopen.com/books/dental-caries-diagnosis-prevention-and-management /dental-caries-etiology-and-remedy-through-natural-resources, accesat in iul.2019

180. Khushwant S. Bhullar and H. P. Vasantha Rupasinghe, "Polyphenols: Multipotent Therapeutic Agents in Neurodegenerative Diseases," Oxidative Medicine and Cellular Longevity, 2013; Article ID 891748, 18 pages. https://doi.org/10.1155/2013/891748.
181. Ashok K. Tiwari, Antioxidants: New-generation therapeutic base for treatment of polygenic disorders, Current Science, 2004; 86 (8): 1092-1102
182. Weckesser. S., Engel. K., Simon-Haarhaus. B., Wittmer. A., Pelz. K., & Schempp, C.M., Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. Phytomedicine , 2007; 14: 508- 516.
183. Pompilio A, Riviello A, Crocetta V et al., Evaluation of antibacterial and antibiofilm mechanisms by usnic acid against methicillin-resistant Staphylococcus aureus. Future Microbiol 2016; 11:1315–1338
184. Valadbeigi T., Chemical Composition and Enzymes Inhibitory, Brine Shrimp Larvae Toxicity, Antimicrobial and Antioxidant Activities of *Caloplaca biatorina*, Zahedan J Res Med Sci. 2016; 18(11):e4267
185. ***American Cancer Society, „What are the key statistics about oral cavity andoropharyngealcancers"www.cancer.org/cancer/oralcavityandoropharyngealcancer/detailedguide/oral-cavity-and-oropharyngeal-cancer-key-statistics. Accesat, noiembrie 2018.
186. Ozdemir, D., Dental caries: The most common disease worldwideand preventiv strategies, Int. J. Microbiol. Res. Rev. , 2017; 6 (5): 340-344
187. Saeed M, Sattari M, Yeganeh F, Sarira Shahnavaz, Correlation between Gingival Expression of STAT1 and Chronic Periodontitis, Int J Dentistry Oral Sci. 2015; 2(10), 158-162
188. Olteanu Z., Oprică L., Truță E., Zamfirache M.M., Behaviour of antioxidative enzymes and of soluble protein in wheat seedlings after lead-induced stress, Analele științifice ale Universității „Alexandru Ioan Cuza", Secțiunea Genetică și Biologie Moleculară, 2011, TOM XII: 75-85.
189. Mates, MJ., et.al, Antioxidant Enzymes and Human Diseases, Clinical Biochemistry, 1999; 32 (8): 595–603
190. Somogyi A., Rosta K., Pusztai P., Tulassay Z., Nagy G., Antioxidant measurements, Physiol. Meas., 2007; 28: R41.
191. Antolovich M., Prenzler P.D., Patsalides E., McDonald S., Robards K., Methods for testing antioxidant activity. Analyst, 2002; 127: 183.
192. Shull S, Heintz NH, Periasamy M, et al. , Differential regulation of antioxidant enzymes in response to oxidants. J Biol Chem 1991; 266: 24398–24403.
193. Amzoiu DC et al, Blood superoxide dismutase activity in patients with knee osteoarthritis, Farmacia, 2014; 62 (3): 460-466
194. Perry JJP, Shin DS, Getzoff ED, Tainer JA., The structural biochemistry of the superoxide dismutase, Biochim. Biophys. Acta, 2010; 1804: 245-262.
195. Mahmoud Hussein Hadwan, Simple spectrophotometric assay for measuring catalase activity in biological tissues , Biochemistry 2018; 19:7 https://doi.org/10.1186/s12858-018-0097-5
196. J. Kaushal et al , Catalase Enzyme: Application in Bioremediation and Food Industry. Biocatalysis and Agricultural Biotechnology, 2018; 16 : 192–199 Available from: https://www.researchgate.net/publication/327052550 [accessed Jul 14 2019].

197. Glorieux C, Calderon PB., Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach, abstract, Biol Chem., 2017; 398(10):1095-1108.
198. Glorieux C, Calderon PB., Regulation of catalase expression in healthy and cancerous cells., abstract, Free Radic Biol Med., 2015; 87:84-97.
199. Chelikani,P., Fita I.,Loewen, PC, Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences, 2004; 61: 192-208.
200. Nicholls, P., Fita, I., Loewen, PC, Enzymology and structure of catalases. Advances in Inorganic Chemistry, 2001; 51: 51-106.
201. Imlay , JA, Pathways of oxidative damage, Annual Review of Microbiology, 2003; 57:395-418.
202. Veitch NC, Horseradish peroxidase: a modern view of a classic enzyme, Phytochemistry 2004; 65: 249-259.
203. Azevedo AM, Martins VC, Prazeres DMF, Vojinović V, Cabral JMS, et.al., Horseradish peroxidase: a valuable tool in biotechnology, Biotechnol Ann Rev Elsevier 2003; 9: 199-247.
204. Shivakumar, A, et.al., Role of Peroxidase in Clinical Assays: A Short Review, Journal of Clinical Nutrition & Dietetics, 2017; 3 (2): 14
205. Pandey VP, Awasthi M, Singh S, Tiwari S, Dwivedi UN, A Comprehensive Review on Function and Application of Plant Peroxidases, Biochem Anal Biochem, 2017; 6: 308.
206. Bernroitner M, Zamocky M, Furtmeuller PG, Peschek GA, Obinger C , Occurrence, phylogeny, structure, and function of catalases and peroxidases in cyanobacteria. J Exp Bot , 2009; 60: 423-440.
207. Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, et al., Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot , 2002; 53: 1305-1319.
208. Wang,Y., Branicky,R., Noë, A. and Hekimi,S., Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling, J. Cell Biol. 2018; 217 (6) 1915–1928
209. Bania, I., Mahanta, R., Evaluation of peroxidases from various plant sources, International Journal of Scientific and Research Publications, 2012; 2 (5)
210. Zeenat Ayoub, Z., Mehta, A., Mishra,S.K., Ahirwal, L., Medicinal Plants as Natural Antioxidants: A review, Journal of Botanical Society, 2017;.48, ISSN 2229-7170
211. Mates, M.J.,et.al., Antioxidant Enzymes and Human Diseases, Clinical Biochemistry, 1999; 32 (8): 595–603
212. Nagar, A., Sharma, V., & Chhipa, A. S. , Role of antioxidants in biological system, a review, Mintage Journal of Pharmaceutical&Medical Sciences , 2017; 6(1): 7-12.
213. Zahin, M., Aqil, F., & Ahmad, I. , The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants.International Journal of pharmacy and pharmaceutical Sciences, 2009; 1(1): 88-95
214. Haffajee, A. D. , Socransky, S. S., Patel, M. R. , Song,X., "Microbial complexes în supragingival plaque," Oral Microbiology and Immunology, 2008; 23 (3) 196–205
215. Dosseva-Panova,VT, Popova, CL, Panov, VE, Subgingival microbial profile and production of proinflammatory cytokines in chronic periodontitis, Folia Medica, 2014; 56. (3), 152–160
216. Kumar PS, Leys EJ, Bryk JM, Martinez FJ, Moeschberger ML, Griffen AL. Changes in periodontal health status are associated with bacterial community shifts as assessed by quantitative 16S cloning and sequencing. J Clin Microbiol. 2006 Oct;44(10):3665-3673.

217. Griffen AL, Beall CJ, Campbell JH, Firestone ND, Kumar PS, Yang ZK, Podar M, Leys EJ. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J. 2012;6(6):1176-1185.
218. Ananthi,R.,Tinabaye,A.,Selvaraj,G., Antioxidant study of usnic acid and its derivative usnic acid diacetate, IJRET: International Journal of Research in Engineering and Technology, 2015; 4(2), eISSN: 2319-1163 | pISSN: 2321-7308
219. Minić I, Pejčić A., Oral Cancer, Incidence and Management, J Oral Cancer Res. 2018; 2(1):23-27
220. El-Naggar AK, Chan J, Grandis JR, Takata T, Slootweg PJ., WHO Classification of Head and Neck Tumours. Lyon: International Agency for Research on Cancer (IARC); 2017.
221. Tanaka TI, Alawi F. Human Papillomavirus and Oropharyngeal Cancer. Dent Clin North Am 2018;62:111-120.
222. Perks, A., Forna, D., Barreira, E., Forna, N., Fricain, J.C., et al, Oral Cancer Prevention., Workshop on Prevention of Oral Cancer in Romania 2019. https://www.researchgate.net/publication/332858570 [accessed Jul 16 2019].
223. Chaturvedi, P., Singh, A., et al., Tobacco related oral cancer, BMJ 2019; 365:l2142 doi: 10.1136/bmj.l2142
224. Bucur, A., et. al., Compendiu de chirurgie oro-maxilo-facială - Vol 2., Q Med Publishing, Bucuresti, 2009, ISBN 978-973-88553-7-3
225. Bucur, A. et al., Ghid de practică în chirurgia oro-maxilo-facială, 2010
226. Flint PW, Haughey BH, Lund VJ, et al, eds. Cummings Otolaryngology, Head & Neck Surgery, Fifth Edition, ed. Philadelphia, PA: Mosby Elsevier, Copyright ©2010
227. Leela K, Devi A. C. Isolation, Purification and Application of Secondary Metabolites from Lichen *Parmelia Perlata*. Biosci Biotech Res Asia 2017;14(4).
228. Malíček, J., Bouda, F., Liška,J., Palice, Z.,& Peksa,O., Contribution to the lichen biota of the Romanian Carpathians, Herzogia 2015; 28 (2) : 713–735
229. Vicol, I. - Preliminary Study On Epiphytic Lichens As An Indicator Of Environmental Quality In Forests From Around Bucharest Municipality (Romania), Analele Universităţii din Oradea - Fascicula Biologie, 2010; Tom. XVII / 1 : 200-207
230. Christensen N.S., Lichens of *Picea abies* forests in Greece, Herzogia , 2018; 31 (1): 219–230
231. Tõrra T. and Randlane, T., The lichen genus *Usnea* (lichenized *Ascomycetes, Parmeliaceae*) in Estonia with a key to the species in the Baltic countries, The Lichenologist, 2007, 39(45): 415-438
232. Nascimbene, J., Marini, L., Motta, R. & Nimis, P. L. Influence of tree age, tree size and crown structure on lichen communities in mature Alpine spruce forests, Biodiversity and Conservation, 2009; 18: 1509–1522.
233. Bucur, L., Istudor, V., Sava, C., Jianu, L., Vameșu, S., Analiza farmacognostică, instrument de determinare a identității,purității si calității produselor vegetale, Editura Ovidius University Press, Constanța, 2002: 52-56
234. *** European Pharmacopoeia 9.0 Edition, Volume 1, EDQM Council of Europe, Strassbourg, 2017;
235. ***Romanian Pharmacopoeia, X ed. Medicală, Publishing house, Bucharest, 1993; (available in Romanian).

236. *** United States Pharmacopeia and National Formulary (USP 30-NF 25), Rockville, Md, United States Pharmacopeia Convention, 2007; 28
237. ***WHO monographs on selected medicinal plants - 2007, Vol. 3.
238. *** WHO monographs on selected medicinal plants – 2014, Vol. 2.
239. Yang, M., Wang, X., Liu, D. et al., Evaluation of edible and medicinal lichen resources in China, Mycosystema , 2018; 37(7): 819-837
240. Pandey, N., Pandey-Rai S., Biochemical Activity and Therapeutic Role of Antioxidants in Plants and Humans, in book Plants as a Source of Natural Antioxidants (ed. N.K. Dubey) CAB International 2015; chapter X : 191-224
241. Plsíkova J.et al., Lichen secondary metabolites as DNA-interacting agents, Toxicology in Vitro, 2014; 28: 182–186
242. Cardarelli, M., Serino, G., Campanella, L., Ercole, P., De Cicco-Nardone, F., Alesiani, O., Rossiello, F., Antimitotic effects of usnic acid on different biological systems. Cell. Mol. Life Sci., 1997; 53: 667–672.
243. Russo A, Piovano M, Lombardo L, Garbarino J, Cardile V. 2008.Lichen metabolites prevent UV light and nitric oxide-mediated plasmid DNA damage and induce apoptosis in human melanoma cells, Life Sci 2008; 83: 468-474
244. Brisdelli, F. et al, Cytotoxic Activity and Antioxidant Capacity of Purified Lichen Metabolites: An In Vitro Study, Phytother. Res., 2012; DOI: 10.1002/ptr.4739
245. De Carvalho, EAB et al., Effect of usnic acid from the lichen Cladonia substellata on Trypanosoma cruzi, in vitro: an ultrastructural study, Micron , 2005; 36: 155–161
246. Zovko M, Kosalec I, Kalodjera Z, Partl A, Pepeljnjak S., Characteristics, chemistry and use of lichens, Farm Glasnik (Croatia) 2007; 63:227-243.
247. Blanquer-Rosselló, M. Hernández-López, R. RocaJordi, P. AdamoValle, O., Resveratrol induces mitochondrial respiration and apoptosis in SW620 colon cancer cells, Biochimica et Biophysica Acta (BBA) - General Subjects, 2017; 1861(2): 431-440
248. McGahon AJ, Martin SJ, Bissonnette RP, et al., The end of the cell line: methods for the study of apoptosis in vitro, Methods Cell Biol, 1995; 46: 153-185.
249. Boustie JB, Tomasi S, Grube M. 2011. Bioactive lichen metabolites: alpine habitats as an untapped source, Phytochem Rev , 2011; 10: 287-307
250. Thirunalasundari et al., Evaluation of antioxidant activity among the epiphytic lichens of Kodaikanal, World Journal of Pharmacy and Pharmaceutical Sciences, 2017; 6(11): 828-841
251. Ullah A. et al., Biological evaluation, characterization and distribution of some lichens of Himalayan region, Pakistan, Journal of Animal and Plant Sciences, 2015; 25(3):580-586
252. Shukla P., Upreti D.K., Tewari L.M., Secondary metabolite variability in Lichen genus *Usnea* in India: A potential source for bioprospection, Journal Of Environmental Science and Technology 2015, 2(4): 44-55
253. *** EMEA, ICH Topic Q 2 (R1)/ CPMP/ICH/381/95, 1995, p.13
254. Conti, M., Pino, A. , Botre,`F. , Bocca, B. , Alimonti, A., Lichen *Usnea barbata* as biomonitor of airborne elements deposition in the Province of Tierra del Fuego (southern Patagonia, Argentina), Ecotoxicology and Environmental Safety, 2009, 72: 1082-1089

255. Carreras, H.A., Pignata, M.L., Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens. Environ. Pollut., 2002; 117: 77–87
256. Conti, M.E., Tudino, M.B., Stripeikis, J., Cecchetti, G., 2004. Heavy metal accumulation in the lichen *Evernia prunastri* transplanted at urban, rural and industrial sites in Central Italy. J. Atmos. Chem., 2004; 49: 83–94
257. Bargagli, R., Nimis, P.L., Guidelines for the use of epiphytic lichens as biomonitors of atmospheric deposition of trace elements. In: NATO Science Series,IV: Earth and Environmental Sciences, 2002; 7: 295–299.
258. Freitas, M.C., Pacheco, A.M.G., Bioaccumulation of Cobalt in *Parmelia sulcata*., J. Atmos. Chem., 2004; 49: 67–82.
259. Golubev, A.V., Golubeva, V.N., Krylov, N.G., Kuznetsova, V.F., Mavrin, S.V., Aleinikov, A.Yu., Hoppes, W.G., Surano, K.A., On monitoring anthropogenic airborne uranium concentrations and 235U/238U isotopic ratio by lichen-bio-indicator technique. J. Environ. Radioact. 2005; 84: 333–342.
260. Ekor, M., The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, Frontiers in pharmacology, 2013; 4
261. Basnet et al., Diversity of Anticancer and Antimicrobial Compounds from Lichens and Lichen derived Fungi: A Systematic Review (1985-2017), Current Organic Chemistry, 2018; 22: 1-14
262. Ingolfsdottir K., Molecules of interest: usnic acid, Phytochemistry, 2002; 61: 729–736.
263. Kristmundsdottir T, Aradottir HA, Ingolfdottir K et al., Solubilization of the lichen metabolite usnic acid for testing in tissue culture. Pharm Pharmacol , 2002;54:1447–1452
264. Dumitrascu, M., *Artemia salina*, Balneo-Research Journal, 2011; 2 (4)11-14
265. S. Michael, S., Thompson, GC et al, *Artemia salina* as a Test Organism for Bioassay, Science, 1956; 123 (3194) : 464
266. Bruno S. Nunes & all., Use of the genus *Artemia* in ecotoxicity testing. Environmental pollution 2006; 144: 453-462.
267. Dvořák P., Žďárský M., Beňová K., Possibilities of alternative generation II biotests at *Artemia*. Interdisc Toxicol., 2009; 2: 45–47.
268. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L. , Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 45: 31-34.
269. Michael, A. S., Thompson, C. G., Abramovitz, M., *Artemia salina* as a test organism for bioassay. Science, 1956; 123: 464.
270. Solý´s, P. N., Wright, C. W., Anderson, M. A., Gupta, M. P. and Phillipson, J. D. (1993). A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. 1993; 59: 250-252.
271. Togulca, M. -The Short-Term Toxicity of Two Toxicants to *Artemia* Nauplii, Tr. J. of Zoology. 1998; 22:259-266.
272. Mohammad Abu Basma Rajeh, Yuet Ping Kwan, Zuraini Zakaria, Lachimanan Yoga Latha, Subramanion L Jothy, Sreenivasan Sasidharan - Acute toxicity impacts of *Euphorbia hirta* L. extract on behavior, organs body weight index and histopathology of organs of the mice and *Artemia salina*, Pharmacognosy Research, 2012, 4 (3)

273. Milhem MM, Al-Hiyasat AS, Darmani H. Toxicity testing of restorative dental materials using brine shrimp larvae (*Artemia salina)*. J Appl Oral Sci 2008; 16:297-301.

274. Sahgal G, Ramanathan S, Sasidhara S, Mordi M, Ismail S, Mansor SM. Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. Seed methanolic extract., Phcog Res 2010; 2:215-20.

275. Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD., A microwell cytotoxicity assay using *Artemia salina*. Planta Med 1993; 59:250 Prior MG. Evaluation of brine shrimp Acetylcholinesterase and butyrylcholinesterase inhibitory compounds from (*Artemia salina*) larvae as a bioassay for mycotoxins in animal feed stuffs, J Comp Med 1979; 43:352-355.

276. Harwig J, Scott P. Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins, Appl Microbiol , 1971;21:1011-1016.

277. Rabelo TK, Zeidán-Chuliá F, Paulo Almeida JP ,et.al. Redox characterization of usnic acid and its cytotoxic effect on human neuron-like cells (SH-SY5Y), / Toxicology in Vitro 2012; 26: 304–314

278. Paudel B. et al, Antioxidant, Antibacterial activity and Brine shrimp toxicity test of some Mountainous Lichens from Nepal, Biol Res, 2012; 45(4), 387-391

279. Ravagliaa, LM, Gonçalvesa, K., Oyamaa,NM, Coelhoa,RG, Spielmannband AA, Hondaa,NK, In vitro radical-scavenging activity, toxicity against *A. salina*, and Nmr profiles of extracts of lichens collected from Brazil and Antarctica, Quim. Nova, 2014; 37, (6) 1015-1021.

280. Martins,M., Silva, M., Silva, L., Lima,V., Pereira,E., Falcão, EP, Melo, A., Henrique da Silva, N., Usnic Acid Potassium Salt: An Alternative for the Control of Biomphalaria glabrata, PLoS One. 2014; 9(11)

281. Veeramani T., Santhanam P., Manickam N., Rajthilak C., Introduction to *Artemia* Culture. 2019; (In: Santhanam P., Begum A., Pachiappan P. (eds) Basic and Applied Zooplankton Biology, Springer, Singapore)

282. ***ATCC, Product Sheet CAL 27 (ATCC® CRL¬2095™)

283. Jiang L1, Ji N, Zhou Y, Li J, Liu X, Wang Z, Chen Q, Zeng X., CAL 27 is an oral adenosquamous carcinoma cell line, Oral Oncol. 2009 Nov;45(11):e204-7.

284. Mayorga, P., Pérez, K.R.; Cruz, S.M., ; Cáceres, A., Comparison of bioassays using the anostracan crustaceans *Artemia salina* and *Thamnocephalus platyurus* for plant extract toxicity screening, Rev. bras. farmacogn.2010; 20 (6); http://dx.doi.org/10.1590/S0102-695X2010005000029

285. Mosmann T., Rapid colorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays, Journal of Immunological methods, 1983; 65:55-63.

286. Stockert J.C., Blazquez-Castro, A., Canete, M., Horobin ,R.W., Villanueva, A., MTT assay for cell viability: intracellular localization of the formazan product is in lipid droplets. Acta Histochem. 2012; 114: 785-796.

287. Alexandrino, C.A. et al, Antitumor effect of depsidones from lichens on tumor cell lines and experimental murine melanoma, Revista Brasileira de Farmacognosia, 2019; https://doi.org/10.1016/j.bjp.2019.04.005

288. Nasri, H.; Bahmani, M.; Shahinfard, N.; Nafchi, A.M.; Saberianpour, S.; Kopaei, M.R. Medicinal plants for the treatment of acne vulgaris: A review of recent evidences. Jundishapur J. Microbiol. 2015, 8, e25580.

289. Karalti,I., Çobanoğlu, G., et.al., Evaluation of antimicrobial activity of the lichens *Physcia aipolia, Xanthoria parietina, Usnea florida, Usnea subfloridana* and *Melanohalea exasperata,* Modern Phytomorphology, 2016; 10: 19–24
290. Emsen B, Aslan A, Turkez H, Taghizadehghalehjoughi AT, Kaya A. The anti-cancer efficacies of diffractaic, lobaric, and usnic acid:In vitro inhibition of glioma. J Can Res Ther 2018;14:941-951.
291. Aminian M., Nabatchian F., Vaisi-Raygani A., Torabi M., Mechanism of Coomassie Brilliant Blue G-250 binding to cetyltrimethylammonium bromide: An interference with the Bradford assay. Analytical Biochemistry, 2013; 434(2): 287-291.
292. Ari F., Aztopal N., Oran S., Bozdemir S., Celikler S., Ozturk S., Ulukaya E. *Parmelia sulcata* Taylor and *Usnea filipendula* Stirt induce apoptosis-like cell death and DNA damage in cancer cells. Cell Prolif, 2014; 47: 457-64.
293. Artenie V., Ungureanu E., Negură Anca Mihaela, Metode de investigare a metabolismului glucidic şi lipidic, 2008; Editura Pim, Iaşi: 97-99, 100-102, 108-110.
294. Bačkorová M., Jendželovský R., Kello M., Bačkor M., Mikeš J., Fedoročko P. 2012. Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines, Toxicol in Vitro, 2012; 26: 462-68.
295. Bacso Z., Everson R.B., Eliason J.F., The DNA of Annexin V-binding apoptotic cells is highly fragmented., Cancer Res, 2000; 60: 4623-4628.
296. Bazin M.A., Le Lamer A.C., Delcros J.G., Rouaud I., Uriac P., Boustie J., Corbel J.C., Tomasi S., Synthesis and cytotoxic activities of usnic acid derivatives. Bioorg Med Chem, 2008; 16: 6860-6.
297. Postelnicu T., Probit Analysis, in Lovric M. (eds) International Encyclopedia of Statistical Science, 2011; Springer, Berlin, Heidelberg, https://doi.org/10.1007/978-3-642-04898-2_461
298. Brandão L.F., Alcantara G.B., Matos M.F.C., Bogo D., Freitas D.S., Oyama N.M., Honda N.K., Cytotoxic Evaluation of Phenolic Compounds from Lichens against Melanoma Cells. Chem Pharm Bull, 2013; 61: 176-83.
299. Brisdelli F., Perilli M., Sellitri D., Piovano M., Garbarino J.A., Nicoletti M., Bozzi A., Amicosante G., Celenza G., Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an in vitro study. Phytother Res., 2013; 27(3): 431-7.
300. Bugni T.S., Andjelic C.D., Pole A.R., Rai P., Ireland C.M., Barrows L.R., Biologically active components of a Papua New Guinea analgesic and anti-inflammatory lichen preparation. Fitoterapia, 2009; 80: 270-273.
301. Chen S., Dobrovolsky V.N., Liu F., Wu Y., Zhang Z., Mei N, Guo L., The role of autophagy in usnic-acid induced toxicity in hepatic cells. Toxicol Sci, 2014; 14: 33-40
302. Darzynkiewicz Z., Bruno S., Del Bino G., Gorczyca W., Hotz M.A., Lassota P., Traganos F., Features of apoptotic cells measured by flow cytometry. Cytometry, 1992; 13, 795-808.
303. Disoma C., Erkisa M., Oran S., Alioglu I., Ulukaya E. Ari F., Usnea filipendula Induces Apoptosis in Human Colon Cancer Cell Lines. Indian J Pharm Sci, 2018;80(6): 1078-1085.
304. Fernández-Moriano C., González-Burgos E., Divakar P.K., Crespo A., Gómez-Serranillos M.P. , Evaluation of the Antioxidant Capacities and Cytotoxic Effects of Ten Parmeliaceae Lichen Species. Evid Based Complement Alternat Med. 2016; http://dx.doi.org/10.1155/2016/3169751.
305. Fried J., Perez A.G., Clarkson B.D., Flow cytofluorometric analysis of cell cycle distributions using

propidium iodide. Properties of the method and mathematical analysis of the data. J Cell Biol 1976; 71, 172-181.

306. Kasimoğullari S.C., Oran S., Ari F., Ulukaya E., Aztopal N., Sarimahmut M., Öztürk Ş. Genotoxic, cytotoxic, and apoptotic effects of crude extract of *Usnea filipendula* Stirt. in vitro. Turk J Biol, 2014;38: 940-47.
307. Mayer M., O'Neill M.A., Murray K.E., Santos- Magalhães N.S., Carneiro- Leão A.M., Thompson A.M., Appleyard V.C. 2005. Usnic acid: a nongenotoxic compound with anti-cancer properties. Anticancer Drugs,2005; 16: 805-9.
308. Nguyen T.T., Yoon S., Yang Y., Lee H.B., Oh S., Jeong M.H., Kim J.J., Yee S.T., Crişan F., Moon C., Lee K.Y., Kim K.K., Hur J.S., Kim H., Lichen secondary metabolites in *Flavocetraria cucullata* exhibit anti-cancer effects on human cancer cells through the induction of apoptosis and suppression of tumorigenic potentials. PloS One, 2014; 9:e111575.
309. Perveen S. and Al-Taweel M., Phenolic Compounds from the Natural Sources and Their Cytotoxicity, 2017; in Phenolic Compounds - Natural Sources, Importance and Applications, Marcos Soto-Hernandez, Mariana Palma-Tenango and Maria del Rosario Garcia-Mateos, IntechOpen, DOI: 10.5772/66898.
310. Pereira C.G., Meireles M.A.M. Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food Bioprocess Technol., 2010; 3: 340–372.
311. Pozarowski P., Grabarek J., Darzynkiewicz Z. , Flow Cytometry of Apoptosis. In Current Protocols in Cytometry, (Hoboken, NJ, USA: John Wiley & Sons, Inc.), 2003; Chapter 7, Unit 7.19.
312. Rieger A.M., Nelson K.L., Konowalchuk J.D., Barreda D.R., Modified Annexin V/Propidium Iodide Apoptosis Assay For Accurate Assessment of Cell Death. J. Vis. Exp., 2011; 50, e2597.
313. Scandalios, J.G. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defences. Braz. J. Med. Biol. Res., 2005; 38: 995-1014.
314. Momoh M.A, Adikwu M.U., Evaluation of the effect of colloidal silver on the antibacterial activity of ethanolic extract of the lichen *Parmelia perlata*. Afri J Pharm and Pharmacol. 2008;2(6):106-109.
315. Su Z.Q., Mo Z.Z., Liao J.B., Feng X.X., Liang Y.Z., Zhang X., Liu Y.H., Chen X.Y., Chen Z.W., Su Z.R., Lai X.P., Usnic acid protects LPS-induced acute lung injury in mice through attenuating inflammatory responses and oxidative stress. International Immunopharmacology, 2014; 22(2): 371–378.
316. Laville N., Aït-Aïssa S., Gomez E., Casellas C., Porcher J.M., Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes. Toxicology,2004, 196(1-2): 41-55.
317. Benson H.J. , Microbiological Applications. A Laboratory Manual în General Microbiology, Published by WCB Publishers, 1990, 53
318. Stockert J.C., Blazquez-Castro A., Canete M., Horobin R.W., Villanueva A., MTT assay for cell viability: intracellular localization of the formazan product is in lipid droplets. Acta Histochem., 2012; 114, 785e796.
319. Cann A.J. - Maths from scratch for biologists. Jon Willey & Sons Ltd.2002
320. Vermes I., Haanen C., Reutelingsperger,C. 2000. Flow cytometry of apoptotic cell death. J Immunol Methods, 2000; 243, 167-190.

321. Vermes I., Haanen C., Steffens-Nakken H., Reutelingsperger C., A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. J Immunol Methods, 1995; 184, 39-51.
322. Walsh J.G., Cullen S.P., Sheridan C., Lüthi A.U., Gerner C. Martin SJ. Executioner caspase-3 and caspase-7 are functionally distinct proteases. Proc Natl Acad Sci USA, 2008;105: 12815-19.
323. Nguyen V.K. et. al, Synthesis and cytotoxic evaluation of usnic acid benzylidene derivatives as potential anticancer agents, Natural ProductResearch, 2019; DOI: 10.1080/14786419.2019.1639176
324. Aminian M., Nabatchian F., Vaisi-Raygani A., Torabi M. 2013. Mechanism of Coomassie Brilliant Blue G-250 binding to cetyltrimethylammonium bromide: An interference with the Bradford assay. Analytical Biochemistry, 2013; 434(2): 287-291.
325. Antolovich M., Prenzler P.D., Patsalides E., McDonald S., Robards K. 2002. Methods for testing antioxidant activity. Analyst, 2002; 127: 183.
326. Hussain M ,Masood S.R, farooq U , Bakhsh H ,Majeed A and Aziz A. In vitro Antimicrobial Potential of Lichen (*Parmelia perlata*) against different Pathogenic Microbes. *International Journal of Pharma Sciences*. 2014;4(4):666-670
327. Talmaç, AC&Çalişir M, Antioxidants and Periodontal Diseases, 2019; DOI: 10.5772/intechopen.81815
328. Bačkorová M., Jendželovský R., Kello M., Bačkor M., Mikeš J., Fedoročko P. Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. Toxicol in Vitro, 2012; 26: 462-68.
329. Bacso Z., Everson R.B., Eliason J.F., The DNA of Annexin V-binding apoptotic cells is highly fragmented. Cancer Res 2000; 60, 4623-4628.
330. Bazin M.A., Le Lamer A.C., Delcros J.G., Rouaud I., Uriac P., Boustie J., Corbel J.C., Tomasi S. Synthesis and cytotoxic activities of usnic acid derivatives. Bioorg Med Chem, 2008; 16: 6860-6866.
331. Behera B.C., Verma N., Sonone A., Makhija U. Antioxidant and antibacterial activities of lichen Usnea ghattensis in vitro. Biotechnol Lett., 2005; 27: 991-995.
332. Brandão L.F., Alcantara G.B., Matos M.F.C., Bogo D., Freitas D.S., Oyama N.M., Honda N.K. Cytotoxic Evaluation of Phenolic Compounds from Lichens against Melanoma Cells. Chem Pharm Bull, 2013; 61: 176-83.
333. Brisdelli F., Perilli M., Sellitri D., Piovano M., Garbarino J.A., Nicoletti M., Bozzi A., Amicosante G., Celenza G., Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an in vitro study. Phytother Res., 2013; 27(3): 431-7.
334. Bugni T.S., Andjelic C.D., Pole A.R., Rai P., Ireland C.M., Barrows L.R. Biologically active components of a Papua New Guinea analgesic and anti-inflammatory lichen preparation. Fitoterapia, 2009; 80: 270-273.
335. Chen S., Dobrovolsky V.N., Liu F., Wu Y., Zhang Z., Mei N, Guo L.,The role of autophagy in usnic-acid induced toxicity in hepatic cells. Toxicol Sci, 2014; 14: 33-40.
336. Darzynkiewicz Z., Bruno S., Del Bino G., Gorczyca W., Hotz M.A., Lassota P., Traganos F., Features of apoptotic cells measured by flow cytometry. Cytometry , 1992; 13, 795-808.
337. Disoma C., Erkisa M., Oran S., Alioglu I., Ulukaya E. Ari F. Usnea filipendula Induces Apoptosis in Human Colon Cancer Cell Lines. Indian J Pharm Sci, 2018; 80(6): 1078-1085.

338. Fried J., Perez A.G., Clarkson B.D., Flow cytofluorometric analysis of cell cycle distributions using propidium iodide. Properties of the method and mathematical analysis of the data. J Cell Biol, 1976; 71, 172-181.

339. Liou G.I., Storz P.,Reactive oxygen species in cancer. Free Radic Res., 2010;44(5): 1-31.

340. Nguyen T.T., Yoon S., Yang Y., Lee H.B., Oh S., Jeong M.H., Kim J.J., Yee S.T., Crişan F., Moon C., Lee K.Y., Kim K.K., Hur J.S., Kim H., Lichen secondary metabolites in Flavocetraria cucullata exhibit anti-cancer effects on human cancer cells through the induction of apoptosis and suppression of tumorigenic potentials. PloS One, 2014; 9:e111575.

341. Olteanu Z., Oprică L., Truță E., Zamfirache M.M. , Behaviour of antioxidative enzymes and of soluble protein in wheat seedlings after lead-induced stress Analele științifice ale Universității „Alexandru Ioan Cuza", Secțiunea Genetică și Biologie Moleculară, 2011, TOM XII: 75-85.

342. Pereira C.G., Meireles M.A.M. , Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food Bioprocess Technol., 2010; 3: 340–372.

343. Pozarowski P., Grabarek J., Darzynkiewicz Z., Flow Cytometry of Apoptosis. In Current Protocols in Cytometry, (Hoboken, NJ, USA: John Wiley & Sons, Inc.), 2003; Chapter 7, Unit 7.19.

344. Rieger A.M., Nelson K.L., Konowalchuk J.D., Barreda D.R.Modified Annexin V/Propidium Iodide Apoptosis Assay For Accurate Assessment of Cell Death. J. Vis. Exp., 2011; 50, e2597.

345. Scandalios, J.G. 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defences. Braz. J. Med. Biol. Res., 2005; 38: 995-1014.

346. Somogyi A., Rosta K., Pusztai P., Tulassay Z., Nagy G., Antioxidant measurements, Physiol. Meas.,2007; 28: R41.

347. Su Z.Q., Mo Z.Z., Liao J.B., Feng X.X., Liang Y.Z., Zhang X., Liu Y.H., Chen X.Y., Chen Z.W., Su Z.R., Lai X.P. 2014. Usnic acid protects LPS-induced acute lung injury in mice through attenuating inflammatory responses and oxidative stress. International Immunopharmacology, 2014; 22(2): 371–378.

348. Vermes I., Haanen C., Reutelingsperger, C., Flow cytometry of apoptotic cell death. J Immunol Methods 2000; 243, 167-190.

349. Vermes I., Haanen C., Steffens-Nakken H., Reutelingsperger C., A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. J Immunol Methods 1995; 184, 39-51.

350. Walsh J.G., Cullen S.P., Sheridan C., Lüthi A.U., Gerner C. Martin SJ. Executioner caspase-3 and caspase-7 are functionally distinct proteases. Proc Natl Acad Sci USA, 2008., 105: 12815-19.

351. Rieger A.M., Nelson K.L., Konowalchuk J.D., Barreda D.R.., Modified Annexin V/Propidium Iodide Apoptosis Assay For Accurate Assessment of Cell Death. J. Vis. Exp. 2011; (50), e2597, doi:10.3791/2597.

352. Yang W.et al., Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells (Database issue), Nucl. Acids Res.2013, 41 (1): 955–961

353. Einarsdóttir E. et al Cellular Mechanisms of the Anticancer Effects of the Lichen Compound Usnic Acid, Planta Med 2010; 76(10): 969-974

354. Hosseinimehr S.J., Rostamnezad M., Ghafari-rad, V.. Epicatechin Enhances Anti-Proliferative Effect of Bleomycin in Ovarian Cancer Cell. Res Mol Med. 2013; 1 (3): 25-28.

355. Wang Z., Methotrexate induces DNA damage and inhibits homologous recombination repair in choriocarcinoma cells, OncoTargets and Therapy 2016:9 7115–7122
356. Costantini, D.,et al, Methotrexate, Paclitaxel, and Doxorubicin Radiosensitize HER2 Amplified Human Breast Cancer Cells to the Auger Electron–Emitting Radiotherapeutic Agent 111In-NLS-Trastuzumab, J Nucl Med 2010; 51:477–483
357. Kern S. et al, Combined antitumoral effects of pretubulysin and methotrexate, PSP 2019, 1, 2460
358. Bundesanzeiger (BAnz), Monographien der Kommission E (Zulassungs- und Aufbereitungskommission am BGA f r den humanmed. Bereich, phytotherapeutische Therapierichtung und Stoffgruppe), 1998; Kln: Bundesgesundheitsamt (BGA)
359. The Homeopathic Pharmacopoeia of the United States (HPUS). 1992. Arlington, VA: Pharmacopoeia Convention of the American Institute of Homeopathy.
360. Summers, A. 1998. Ethnobotany of Some Common Lichens of Northern California. Rohnert Park, CA: Unpublished paper prepared for Mycology Biology 339 Course, 1998; Sonoma State University.
361. Balasubramanian M.&Nirmala,P., Evaluation of antioxidant properties of Foliose lichens, Journal of Chemical and Pharmaceutical Research, 2014, 6(9):177-184
362. Eryilmaz, I.E., Guney Eskiler, G., Egeli, U. et al., In vitro cytotoxic and antiproliferative effects of usnic acid on hormone-dependent breast and prostate cancer cells. J Biochem Mol Toxicol. 2018; e22208: 01-08
363. Cronstein, BM, The Mechanism of action of Methotrexate, Rheumatic Disease Clinics of North America, 1997; 23(4): 739-755
364. Longley,BD, et al, 5-Fluorouracil: mechanisms of action and clinical strategies, Nature Reviews Cancer, 2003; 3: 330–338
365. Dorr RT, Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics, Semin Oncol. 1992;19(2):3-8.
366. Wendakoon C., Calderon P., Gagnon D., Evaluation of Selected Medicinal Plants Extracted în Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens. J Med Act Pal 2012, 1, 60-68
367. Nikoli G.S., Zlatkovi S., Nikoli N., Identification and Compatibility of the Major Active Principles în Some New Natural Origin Antiseptics, Russian Journal of Physical Chemistry A, 2009; 83,(9): 1612–1616.
368. Kępa, M., Miklasińska-Majdanik,M., Robert D. Wojtyczka, R.D. et al., "Antimicrobial Potential of Caffeic Acid against *Staphylococcus aureus* Clinical Strains," BioMed Research International,2018, Article ID 7413504, 9 pages, https://doi.org/10.1155/2018/7413504
369. Kozyra, M., Biernasiuk, A., Malm, A., Analysis of phenolic acids and antibacterial activity of extracts obtained from the flowering herbs of *Carduus acantoides* L., Acta Poloniae Pharmaceutica ñ Drug Research, 2017; 74 (1): 161-172
370. Panichayupakaranant, P., Tewtrakul, S., Yuenyongsawad, S., Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract, Food Chemistry, 2010; 123: 400–403
371. Sova M., Antioxidant and antimicrobial activities of cinnamic acid derivatives, Mini Rev Med Chem., 2012; 12(8): 749-767.