EXPLORATION OF THE MICROBIAL BIODIVERSITY OF ALBANIAN MEDICINAL PLANTS

R. TROJA\textsuperscript{a,*}, D. PRIFTI\textsuperscript{a}, N. DALANAJ\textsuperscript{a}, A. PETRE\textsuperscript{a}, E. TROJA\textsuperscript{b}, R. CECI\textsuperscript{b}, A. CET\textsuperscript{a}

\textsuperscript{a}Faculty of Natural Sciences, Department of Industrial Chemistry, University of Tirana, Tirana, Albania
\textsuperscript{b}Faculty of Medicine, Department of Pharmacy, University of Tirana, Tirana, Albania
E-mail: rozitroja@yahoo.com; donikaprifti@yahoo.it

Abstract. Natural Albanian habitats are known and studied for the growth of medicinal plants. These environments are considered also as an ‘origin place’, responsible for the microbial populations located in these plants. Albania is a small country, but with very big differences in the relief and climate, sustaining varieties of plants, including medicinal plants, too. There are actually, completed botanic, medicinal and biotechnological studies of them, but not yet detailed studies about their microflora and other microorganisms originated by the surrounding environments and developed together with epiphyte microorganisms. The genera and species isolated from medicinal plants are originated from natural microflora, the pollution of respective environments and also from microbial strains present in the air and soil of the areas where they are grown. Climatic changes and other natural phenomena are responsible for the presence of different microbial strains. Technical operations in order to use original plants as pharmaceutical preparations, for example the treatments at different temperatures, drying processes or chemical manipulations in order to extract chemical compounds with industrial importance, offer another view of the world of microorganisms present on the surfaces of medicinal plants and/or in their specific biological parts. This study is an introduction of a detailed research work for the isolation, identification and characterisation of the microbial strains, obtained from medicinal plants present in the Mediterranean Albanian environments.

Keywords: habitat, medicinal plants, carotenoids, collection of microorganisms.

AIMS AND BACKGROUND

The aim of the scientific research work is to make a full investigation about the microbial world present in the most distributed medicinal plants in Albania. Microbial study of medicinal plants is realised for these purposes:

- to explore and conserve the microbiological strains presented in medicinal plants;
- to make the comparative studies about the total number of microorganisms in different medicinal plants and to discover the relationship between their pres-
ence and chemical substances of the plants, which stimulate or inhibit the presence of specific genera and species (the relationship medicinal plants – microorganisms);

- to isolate and identify new genera and species with a potential use as producers of important compounds: substances with provitaminic activity as carotenes, enzymes, killer-proteins or others;
- to manage the presence of microorganisms in pharmaceutical preparations originated from medicinal plants, in order to control the safety use of them as drugs;
- to assess the presence of foreign strains during technological processes of the preparation of drugs and to reduce the risk from their action;
- to find a position for new isolated strains of bacteria, yeasts and moulds in Albanian Collection of Industrial Microorganisms\textsuperscript{1,2}.

Autochthon medicinal plants selected for the study were \textit{Thymus vulgaris}, \textit{Tilia cordata}, \textit{Laurus nobilis}, \textit{Helichrysum arenarium}, \textit{Matricaria chamomilla}, \textit{Saturea montana}, \textit{Rosa canina}, \textit{Folium sennae} and \textit{Salvia officinalis}\textsuperscript{3–6}. The study on them was based principally in:

- botanical aspects and characteristics;
- technological aspects (drying, packaging, the use of supercritic fluids for the extraction of essential oils, etc.)
- the important role in medicine (curative properties, relationship human being – medicament originated from them, etc.)\textsuperscript{3,5,6}.

The importance of the microbiological studies of these plants was related also with: their role, as a part of autochthon flora; their economic values – plants for exportation; their advantage as essential oil sources, with a variety and complexity of chemical organic compounds – responsible for the properties of these essential oils; the possibility to use them as objects for detailed microbiological investigation and taxonomic studies\textsuperscript{7–10}.

The selection of the samples was made as ‘ready to use’ products, in order to evaluate the microbiological situation before their distribution and use.

**EXPERIMENTAL**

9 medicinal plants (botanic varieties described above in details) taken by ALBDUCROS and FLORIFARMA were selected for taxonomic studies and characterised as follows:

- botanical view;
- chemical structure;
- medical parts;
- botanical parts of them and their pharmacological role;
- detailed impacts;
- indications\textsuperscript{5,6,9}. 

972
The microorganisms included in the same physiological group were determined and evaluated using the method of ‘limited dilutions’ in selective liquid media. This method was used also for all the other strains, which do not grow, or grow slowly in solid artificial media. The experiments were realised in selective media MPA, YEPG, CAPEK, respectively characteristics for the growth of bacteria, yeasts and moulds. Total count of them was evaluated directly in Petri dishes or using ‘the enumeration of the sectors’ in all the cases of a big number of the presented microbial colonies.

An introduction of the whole experimental work is detailed as follows:
• Microscopic studies of the moulds, using the ‘pressed point preparations’ of them were realised.
• Identifications of the strains in genera and species, based on the determination of the general characteristics through morphological studies were finished.
• The autochthon microorganisms were isolated and purified from the samples, in order to use them for the future identifications.
• Comparative studies of the microbial charge, taking into consideration the characteristic properties of the plants in study process and their behaviour with epiphyte microflora and/or other microorganisms, derived from the pollution, specific climatic conditions, dust, wind, technological manipulations, etc. were realised.

Some of the results taken from microbiological analysis of the selected plants are presented in Figs 1–6. Similar graphics were designed for all the microbiological analyses, realised during the experimental work.

Fig. 1. Microbial charge of *Luarus nobilis*
Fig. 2. Microbial charge of *Matricaria chamomilla*

![Fig. 2](image)

Fig. 3. Microbial charge of *Tilia cordata*

![Fig. 3](image)

Fig. 4. Microbial charge of *Thymus vulgaris*

![Fig. 4](image)

Fig. 5. Distribution of the mould species, present in the analysed samples

![Fig. 5](image)
There were observed quantitative and qualitative changes of the microorganisms. There was also monitored the appearance of new species, changes in the morphological and technological characteristics as sporulation, assimilation and fermentation of carbohydrates, flocculation, etc.

The principal parameters which determine the microbial charge of the medicinal plants are: water activity, heat treatments, conservation of plants for a long time, consumption of different parts of the plants, their fruits, their flowers, etc., chemical compounds as alcohols, acids, esters, terpenes, etc.

Water activity is the most important parameter with a direct impact on the microbial charge. Tolerant values of water activity in medicinal plants are responsible for the moulds isolated from them.

The presence of Cladospora and Alternaria, isolated and identified during the experimental work is related with the microbiology of the soil where these plants are grown.

Microbial charge of yeasts is very low. Medicinal plants are not so attractive for facultative aerobe mezophylic species of yeasts, perhaps this is related with the carbon sources present in their cells.

CONCLUSIONS

• Medicinal Albanian plants are rich with specific microorganisms and will serve to isolate new strains with microbiological and biotechnological importance.

• Their structure and their characteristics, environmental conditions too, are in favour of the development of the principal classes of the moulds as Phycomycetes, Ascomycetes and Fungi imperfecti.

• Yeasts are not isolated from dried plants. They are present only in fresh plants, this is related with the high values of water activity.

• The values of water activity are not in favour of the bacterial growth (low than 0.9), so there are identified bacterial strains originated from other sources in contact with the above medicinal plants.

• Each plant, with specific characteristics, is attractive for different species of microorganisms, example Aspergillus is the characteristic microflora of Cessia angustifolia.

• Dominant species of bacteria are Bacillus – pigmented in red and yellow.
• *Satureja montana* and *Cessia angustifolia* offer a high microbial charge, perhaps related with their cell construction or other environmental characteristics. A new experimental plan is constructed to clarify the above tendency.

• The reduced charges of microorganisms are related with bacteriostatic and/or bactericide properties of some of them. Alcohols, terpens and saponins are responsible for the above reduction.

REFERENCES


Received 21 November 2008
Revised 24 December 2008