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Comparative assessment of antibacterial activity of cultivated (*Mentha spicata* var. *viridis*) and wild (*Mentha smithiana*) mint species

Borozan Aurica Breica[1], Popescu Sorina[1*], Moldovan Camelia[1*], Dumbrava Delia[1], Trofim Alina[2], Madoșă E.[1]

[1*]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timișoara, 119 Calea Aradului, RO- 300645,Timisoara,Romania
[2]State University of Moldova, Alexei Mateevici Street 60, Chisinau, Republic of Moldova

*Corresponding author. Email: sorinapopescu@usab-tm.ro; cameliamoldovan@usab-tm.ro

Abstract Over the last decade there are more and more researches involving natural therapies.The plants which are the main instrument in these therapies have beneficial properties to the organism due to the secondary metabolic compounds they synthesize. In this work a comparison has been made between the biological activity of the extracts of the *Mentha spicata var viridis* and *Mentha smithiana*. The bacterial cultures tested were *Escherichia coli* and *Salmonella spp.*, the negative Gram bacteria belonging to the *Enterobacteriaceae* family. The alcoholic extracts were obtained from different parts of the plants (roots, leaves, flowers). The young bacterial suspensions were inoculated on specific nutrient media and the biological potential assessment of mint extracts was carried out by the diffuse method. The data obtained were reported on solvent (ethyl alcohol) and two antibiotics (ampicillin and ceftaxim). Experimental results have shown that alcoholic extracts from the two species of mint may inhibit bacterial growth, but the zones of inhibition are lower than those caused by the two antibiotics. Bacterial cultures have shown a slight sensitivity to the solvent. It may be estimated that flower and leaf extracts from both mint species have biological activity.

Key words

antibacterial activity, *Mentha spicata* var. *viridis*, *Mentha smithiana, Escherichia coli. Salmonella spp.*

Throughout the world, numerous studies emphasize a significant increase in the number of bacterial species that are capable of developing mechanisms for resistance to the action of antimicrobial agents. Concernment is that most researches indicates a progressive trend in levels of microbial resistance [21]. *Escherichia coli* is a common but complex gastro-intestinal bacterium in the form of Gram negative bacilli, produces infections and in the last period has gained increasing resistance to antibiotics [20]. Salmonella species are of high public health interest, as they cause diseases in humans and animals [10] and according to data provided by several authors are responsible for thousands of deaths worldwide [1, 6, 9, 13].

Salmonella spp. together with *Escherichia coli* are part of the family Enterobacteriaceae. *Salmonella* spp. is a Gram negative bacterium rod-shaped, which are optional anaerobic, the vast majority of species are mobile, with some exceptions [11, 19].

The discovery of new antimicrobial substances with different chemical structure and new mechanisms for action in the infectious diseases is a necessity [16]. Infectious diseases have become a major problem in the world, especially in more developed societies [3]. Also, the increasing frequency of micro-organisms in food leads not only to a decrease in the nutritional and organoleptic value of food products, but also to the generation of more toxins that are harmful to human health. Therefore, our studies were focused on microbial species with implications in the food and medical fields.

Today more than once, the scientific world has appealed to alternatives to inhibit the activity of microbial agents that have exceeded the limits imposed by classical medication [12]. The study of plant extracts for the isolation of compounds is a widespread concern in the medical and food world. Thus, a multitude of herbal bio-active extracts or constituents are investigated for their valorisation and the development of effective chemotherapeutic instruments applicable to the management or therapy of infections caused by certain bacterial species [4, 14]. These bio-active products may be phenolic compounds, which form part of essential oils [7] but also tannins.

One of the plants that is an inexhaustible source of such compounds is mint. Mint is part of the Lamamiaceae family, it is a perennial grass with a rich

chemical composition, characterized mainly by high menthol content, used in the food, cosmetic and medical industries [5, 18].
Mint has antibacterial properties. Studies carried out by Dixit (2013) have shown that mint extracts have a higher or lower biological activity depending on the bacterial species. For example, the effect of mint extracts was more pronounced on Gram-positive bacteria compared to Gram-negative bacteria. It was also pointed out that the reaction of bacteria depends on solvent. Even if bacteria are of genetic similarity and are assigned to the same taxonomic group, their response to the same antimicrobial compounds may be different depending on the solvent.
Kazemi et al. (2012) studied the antibacterial and antifungal activity of some medicinal plants, including *Mentha piperita* and *Mentha spicata* and demonstrated that *Mentha piperita* has good antifungal and antibacterial activities.
The same authors pointed out that the antibacterial and antifungal activity of *M. piperita* leaves is due to the tannins and flavonoids in its chemical composition. [8].
It is known that *Escherichia coli* and *Salmonella* spp. also cause gastrointestinal problems. Therefore, it should be mentioned that the usefulness of mint is not limited to certain bacteria and some authors have directed the evaluation of the antimicrobial potential of mint on different gastrointestinal pathogens [15].
Some researchers considered that wild mint does not have the biological potential of cultivated mint. Thus, in this paper we made a comparison between the antibacterial activity of cultivated mint extracts (*Mentha spicata* var. viridis) and the wild one (Mentha smithiana) against *Escherichia coli* and *Salmonella* spp.

Material and Method

Obtaining bacterial suspensions
The behavior of Gram-negative bacterial culture Escherichia coli and Salmonella spp. Was monitored in the presence of the 5 alcoholic extracts of *Mentha spicata* var. viridis si *Mentha smithiana*. The tested enterobacteria were under-cultured on nutrient broth for 24 hours at 37°C.
Obtaining mint extracts
Alcoholic extracts of *Mentha smithiana* (fig. 1A), *and Mentha spicata* var. *viridis* (fig. 1B), were obtained from different parts of the plants by the team of Inorganic and Organic Chemistry disciplines from the Faculty of Food Engineering Timisoara.
For *M. smithiana* 3 extracts were prepared, namely: E2- extract from the roots; E3-extract from leaves and E4-extract from flowers.
For *M. spicata* var. viridis 2 extracts were prepared namely: E5-extract from leaves and E6-extract from flowers. Ethyl alcohol (E1) was used as a control.

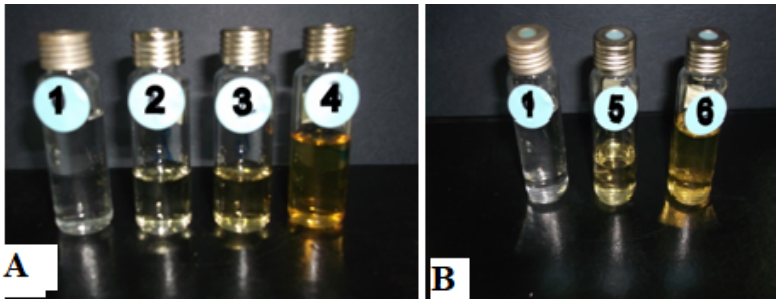

Fig. 1. Alcoholic extracts of *Mentha smithiana* (A) *Mentha spicata* var. *viridis* (B), (original photo)
Legend: 1- ethyl alcohol 96%; 2-mint root extract; 3,5-mint leaf extracts; 4.6-extracts of mint flowers
Sensitivity was reported to two antibiotics: ampicillin (E7) and cefotaxime (E8).

Diffusion method for testing the biological activity of enterobacteria
The testing of alcoholic mint extracts was performed by the diffusimetric method (CLSI). This is a fast and efficient method to establish the simple or multiple resistance of a microorganism to the action of a substance. The advantage of this method is that the degree of sensitivity or resistance to a wide range of substances can be determined.
The 6 mm diameter biodiscs, impregnated with alcoholic mint extracts were placed on the surface of the Mueller Hinton nutrient medium, inoculated with the 2 bacterial cultures. In parallel, the antimicrobial effect of the solvent (control variant: E1) and antibiotics was studied. The microbial suspensions were inoculated in two repetitions. The inoculated Petri dishes were incubated in a thermostat for 24 hours at 37°C. The effect of the extracts was assessed depending on the diameter of the inhibition area (mm).

Results and Discussions

The experimental results obtained from the microorganism – plant alcohol extract interaction were reported to the control variant (E1) and to the two antibiotics. Both bacterial species tested showed a slight sensitivity to solvent (E1), but a greater sensitivity to mint extracts. These results were shown in Figures 2-5.

Effect of mint extracts on the species *Escherichia coli*

From studies carried out so far, it can be claimed that extracts of *Mentha smithana* have a better anti-bacterial activity against micro-organisms involved in human pathology compared with other plants [5].

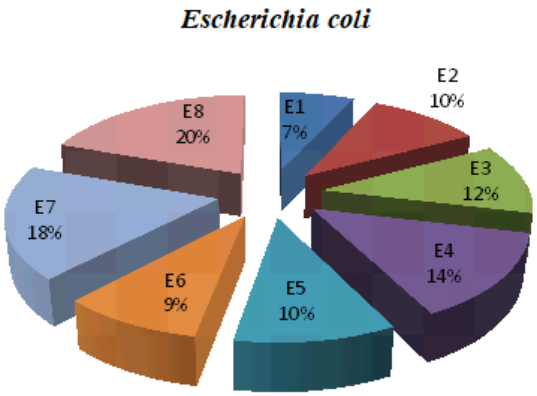

Fig. 2. Effect of extracts of *Mentha smithiana* and *M. spicata* var. viridis on the bacterial species *E. coli*
Legend: E1 - ethyl alcohol; alcoholic extracts of *M. smithiana*: E2- root extract, E3-leaf extract; E4 - flower extract; alcoholic extracts of *M. spicata* var. *viridis*: E5- leaf extract; E6-flower extract; antibiotics: E7-ampicillin, E8-cefotaxime

Mint extracts inhibit *E. coli* bacteria compared to solvent (E1). Relative to antibiotics (E7, E8), alcoholic mint extracts have a lower inhibitory effect. Depending on the inhibitory activity, the extracts can be placed in descending order as follows: E4>E3>E2, for *M. smithiana* and E5>E6 for *M. spicata* var *viridis* (Fig. 2).

The effect of mint extracts on *Salmonella*

From the results contained in figure 3, it is observed that there are no differences between the control variant (E1) and the E2 extract of *M. smithiana* (fig. 3).

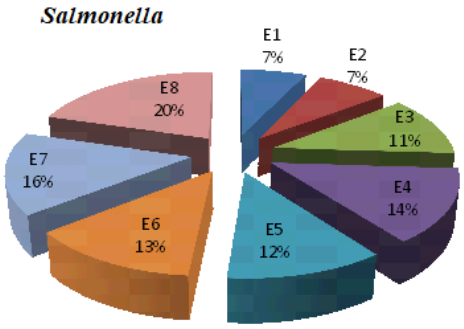

Fig. 3 Effect of *M. smithiana* and *M. spicata* var *viridis* extracts on *Salmonella* bacterial species
Legend: E1 - ethyl alcohol; Alcoholic extracts of *M. smithiana*: E2- root extract, E3-leaf extract; E4 - flower extract; Alcoholic extracts of *M. spicata* var *viridis*: E5- leaf extract; E6-flower extract; Antibiotics: E7-ampicillin, E8-cefotaxime

Salmonella spp. was sensitive to the extracts of leaves (E3) and flowers (E4) of *M. smithiana* and to both extracts of *M. spicata* var *viridis* (E6, E5), (fig. 4B).
The degree of sensitivity of the bacterial species in the presence of the two antibiotics was different, being more accentuated in the case of the antibiotic E8.

Among the tested extracts, E4 of *M. smithiana* and E6 of *M. spicata* var *viridis* showed the highest antimicrobial activity on *Salmonell*a.

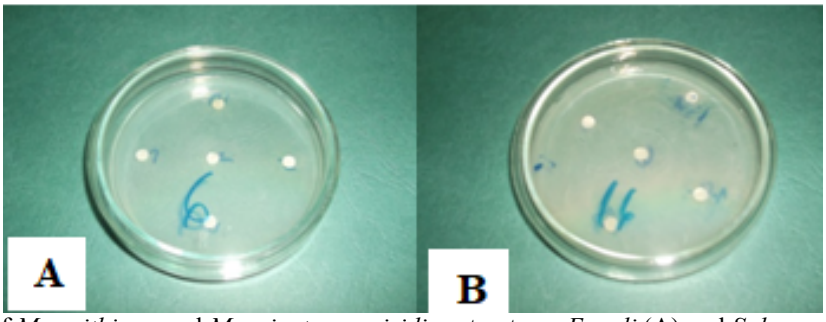

Fig. 4. Effect of *M. smithiana* and *M. spicata* var *viridis* extracts on *E. coli* (A) and *Salmonella* (B) species, (selective, original pictures)

The evaluation of our results pointed out that mint extracts had an inhibitory effect on the Gram-negative bacteria studied, as demonstrated also by Shalayel et al. (2017). They have shown that mint extracts negatively influence the growth of bacterial cultures, but this effect varies depending on the extraction solvent. Ethanol extract has antibacterial activity, but the strongest effect against Gram-negative pathogens is exerted by mint extract in ethyl acetate. Recent research has also shown that the volatile compounds in *Mentha viridis* have remarkable antibacterial and antioxidant properties and could be used in the pharmaceutical and food fields [2].

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Conclusions

The microbial species tested showed a slight sensitivity to the control alcoholic solvent (E1).

Following the antibiogram, it is found that of the two antibacterial antibiotics, E8 stands out, to which the bacteria have a greater sensitivity.

All alcoholic extracts of *M. smithiana* tested, E2, E3, E4 showed antimicrobial activity, but this was more pronounced in the last two extracts.

Both alcoholic extracts of *M. spicata* var *viridis* negatively influenced the bacteria, but the size of the inhibition zone varied depending on the bacterial species.

In conclusion, the use of mint extracts can be recommended, because they have antimicrobial activity against the tested microbial species, noting that the inhibitory effects are not higher compared to antibiotics.

Both plants may also be used with the specification that it is preferable to take into account the component part of the plant from which the extract is obtained and the bacterial species under test. Following the tests, it can be appreciated that the extracts from flowers and leaves have the best biological activity.

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