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Original Contribution

STUDY ON ANTIBACTERIAL ACTIVITY OF HABERLEA RHODOPENSIS

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SUMMARY

The present work is a preliminary study of the antibacterial activity of the total extract of the medicinal plant *Haberlea rhodopensis*. A total extract from *Haberlea rhodopensis* leaves macerated for 48 hours in 70% water-ethanolic solution with subsequent distillation of the ethanol in vacuum vaporizer to a drug/liquid phase proportion of 5:1, was used. The antibacterial activity of the extract was done on some standard and wild pathogenic bacterial strains. The testing was done by the disc-diffusion method using filter paper discs impregnated with the different concentration of the initial extract: undiluted or diluted 1:1 or 1:2. The results show that the inhibition of the bacterial growth was more pronounced on *Staphylococcus aureus* than on Gram –negative strains - *Pseudomonas aeruginosa* and *Escherichia coli*. A possibility of its antibacterial activity is discussed.

Key words: medicinal herb, antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Various medicinal plants have been used for years in daily life to treat diseases all over the world. Medicinal plants are an important therapeutic option for various diseases. According to World Health Organization, medicinal plants are a substantial source of a variety of newer herbal drugs. *Haberlea rhodopensis* belongs to the family *Gesneriaceae* and it is a Balkan endemic relict that is widely distributed mainly in the Rhodope Mountains and some regions of the Sredna gora Mountains and the Balkan Mountains. It represents a rîsette- like evergreen herb, which requires specific soil and climatic conditions for growing. *Haberlea rhodopensis* belongs to the so called ”resurrection plants” due to its ability to fall in anabiosis for a long time when there are unfavourable conditions and to restore back to normal when the conditions become appropriate.

Haberlea rhodopensis is a relatively less explored plant. The basic studies are on the explanation of the mechanism of its anabiosis. It has been proven that in case of desiccation the herb is able to save a part of its water content while the metabolism continues at a very slow pace. There are assumptions that the remaining water forms a thin layer around proteins and thus protects them from denaturation.(1) The role of the lipids and the polysaccharides in the mechanism of anabiosis has been discussed.(2) Other studies are focused mainly on the antioxidant potential and thermo- and photostability of photosynthesis system (3,4,5) of the herb at different conditions. There are no sufficient data about the phytochemical composition in the accessible literature - a significant amount of chlorophyll and some enzymes (superoxiddimutasa, citratdehidrogenasa etc.) have been found. Flavonoids, flavonoid tannin, zeexantin, ascorbate, glutation (6) etc have been found in other species from the *Gesneriaceae family*. There is a lack of information about any antibacterial activity of the extract or separate phytochemical substances from the plant. According to the natives, *Haberlea rhodopensis* has been used for treatment of *Paronichya contagiosa* by adding it to the food of the animals. Additional investigations in specialized literature showed that this is probably a polyaetiologic disease whose current treatment includes different antibacterial

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agents and local antiseptics (fotmalin, cuprum sulfuricum) (7).

Aim

The aim of the present study is to determine the presence of an antibacterial activity of the total extract from *Haberlea rhodopensis*.

MATERIAL AND METHOD

Haberlea rhodopensis was collected from different locations in the Plovdiv region, Bulgaria. The leaves of the plants were dried and roughly pulverised. A total extract from *Haberlea rhodopensis* leaves macerated for 48 hours in 70% water-ethanolic solution with subsequent distillation of the ethanol in vacuum evaporizer to a drug/liquid phase proportion of 5:1, was used. The antimicrobial activity of the extract on standard strains of *Staphylococcus aureus (S. aureus* ATCC 25923*; MSSA S.aureus* ATCC29213 (Methicillin-sensitive), *MRSA S. aureus* ATCC 39592 (Methicillin-resistant), *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853, and clinical isolates of *S. aureus* 707 and 1707, was tested.

The testing was done by the disc-diffusion method using filter paper discs impregnated with 5 mcl of a solution in different concentration of the initial extract: undiluted or diluted 1:1 or 1:2. A 24-hour culture of the examined strains with microbe density of 0.5 on the McFarlan scale was inoculated on Mueller Hinton plates (a standard solid environment for antibiogrammes). *D*iscs impregnated with different concentration of the extract were applied to the surface of Mueller Hinton plates. The inoculated cultures have been incubated for 24 hours at 35^0Ñ. After the incubation the diameter of the zones of bacterial growth inhibition around the discs were measured in mm.

RESULTS AND DISCUSSION

Results are presented on **Table 1** and **Figures 1, 2, 3, 4** and **5**. Our results show that the undiluted and diluted 1:1 and 1:2 *Haberlea rhodopensis* has an antibacterial effect on the test pathogenic strains. This effect is markedly directed towards Gram-positive microorganisms – *S. aureus*, including MSSA and MRSA (**Figures 1.2, 3, 4**). Besides it shows an activity in a smaller degree against some Gram – negative strains such *P. aeruginosa* and *E. coli* (ATCC 25922) (**Figure 5**), which corresponds with the well known fact that the Gram- negative microorganisms have increased activity due to their morphological and other characteristics such as a triple structure of the cell wall (8). The present study shows the antibacterial activity of the extract, but it is impossible to tell for sure whether the extract acts like an antibiotic or an antiseptic.

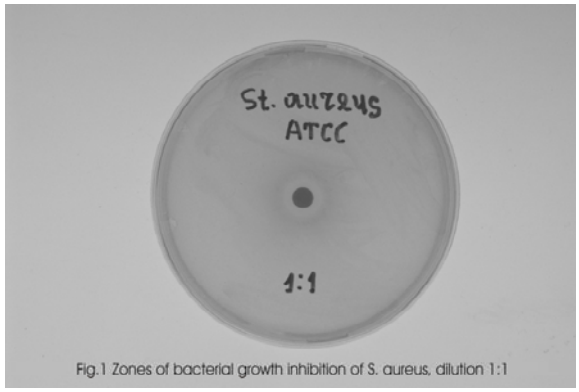

Fig.1 Zones of bacterial growth inhibition of S. aureus, dilution 1:1

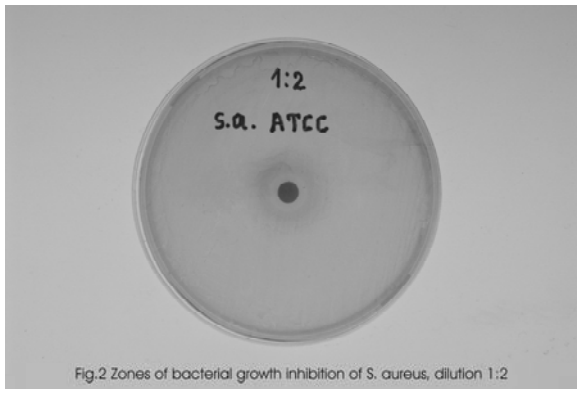

Fig.2 Zones of bacterial growth inhibition of S. aureus, dilution 1:2

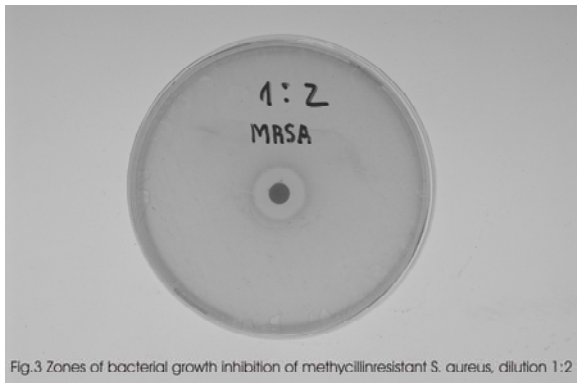

Fig.3 Zones of bacterial growth inhibition of methycillinresistant S. aureus, dilution 1:2

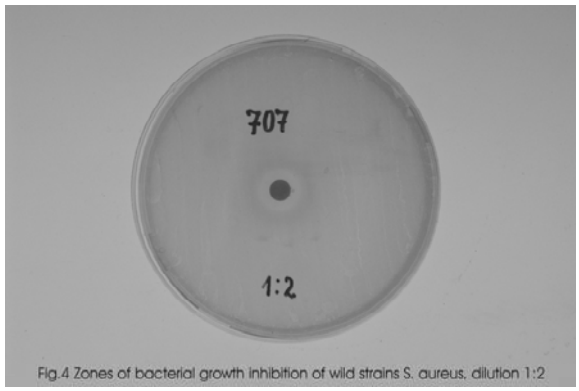

Fig.4 Zones of bacterial growth inhibition of wild strains S. aureus, dilution 1:2

Table 1. *Zones of bacterial growth inhibition in different tested strains (mm)*

Bacterial strains	*Disc-non-dilution extract*	*Disc-dilution extract 1:1*	*Disc-dilution extract 1:2*
S. aureus ATCC 25923	14	10	10
MSSA S. aureus ATCC29213	20	14	12
MRSA S. aureus ATCC39592	18	15	14
S. aureus clinical isolates 707	16	14	12
S. aureus clinical isolates 1707	18	14	12
Å. coli ATCC25922	8	-	-
P. aeruginosa ATCC27853	12	-	-

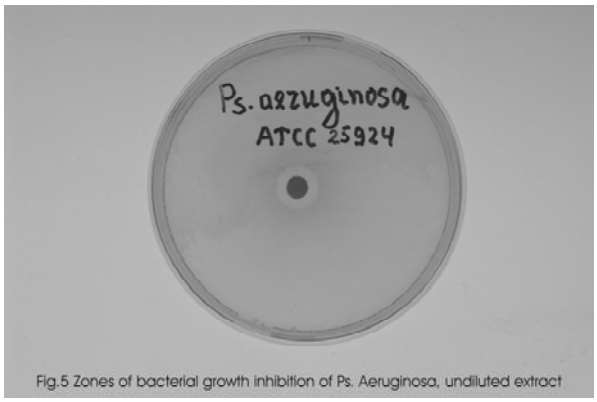

Fig.5 Zones of bacterial growth inhibition of Ps. Aeruginosa, undiluted extract

The present study has preliminary character and can be extended by the determination of the minimal inhibitory concentration (MIC) of the extract.

It should be noted that there are some plants with antibacterial and antiviral activity. Some of them have found their place, especially in the folklore medicine (*Allium sativum, Allium ursinum, Geranium macrorrhizum*), but most herbs with antimicrobial activity have high toxicity (*Veratrum lobelianum, Helleborus odorus, Artemisia vulgaris, Hedera helix* etc.), which restrict their broader use (9, 10).

The discovery and the application of natural antibacterial drugs will play a role in human as well as veterinary medicine in the future. This would help limit the accumulation of synthetic remedies in produced foods like meat, milk, honey etc.

Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore.

Therefore, such plants should be investigated to better understand their properties, safety and efficacy.

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