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ANTIMICROBIAL ACTIVITY OF EXTRACTS OF "HAIRY" ROOT CULTURE AND REGENERATED PLANTS OF *RUTA GRAVEOLENS* L. AGAINST SOME SOIL AND PATHOGENIC BACTERIA

Bielikova Olena[1]*, Matvieieva Nadiia[2], Tashyrev Oleksandr[2], Yastremskaya Larisa[3]

[1]Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]Institute of Cell Biology and Genetic Engineering NAS of Ukraine, Kyiv, Ukraine
[3]National Aviation University, Kyiv, Ukraine

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Ruta graveolens L. has potential value in traditional medicine by various active substances in the composition, which act as antioxidants and have anticancer, antiviral and antibacterial properties. The present study was undertaken to evaluate the effect of genetic transformation on antibacterial activity of ethanol and water extracts of "hairy" root culture and regenerated plant of *Ruta graveolens* against human pathogenic bacteria *Staphylococcus aureus* B-904, *Bacillus subtilis* sub sp. *subtilis*, *Escherichia coli* B-906, *Pseudomonas aeruginosa* B-907 and soil bacteria *Micrococcus luteus*, *Enterobacter hormaechei*, *Kocuria carniphila*, *Citrobacter freundii*, *Rhodococcus erythropolis* and yeast *Saccharomyces cerevisiae*, *Candida tropicalis*. Both extracts from control and transgenic roots demonstrated antimicrobial activity against Gram-positive bacteria. The extracts didn't inhibit growth of Gram-negative bacteria and yeast. The activity of ethanol extract was greater than the activity of water extracts. In some cases, the extracts from 'hairy" root culture exhibited higher antimicrobial activity than the extracts from the control plants.

Keywords: *Ruta graveolens* L.; antimicrobial activity; "hairy" root culture microorganisms

Introduction

Ruta graveolens L. is the source of more than 120 biologically active compounds of different classes such as phenols, flavonoids, alkaloids, anthocyanins, indoles, tannins (Melnyk and Grytsyk, 2015). The plants have potential value in traditional medicine by various active substances in the composition, which act as antioxidants and have anticancer, antiviral and antibacterial properties (Fadlalla et al., 2011; Asgarpanah and Khoshkam, 2012). Previously, it was shown that extracts of *Ruta graveolens* exhibit antimicrobial activity against a broad spectrum of microorganisms (Ivanova et al., 2005; Pandey et al., 2011; Amabye and Shalkh, 2015; Saeidinia et al., 2016).

The plants collected in nature usually are a source of biologically active compounds with antimicrobial activity. At the same time, transgenic plants and "hairy" roots can also be used as a source of the compounds with antimicrobial properties. Therefore, now there is high interest in obtaining transgenic roots of *Ruta graveolens*. "Hairy" root culture can grow in bioreactors in nutrient media

***Corresponding author:** Olena Bielikova, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine, ✉ belikova.e.y@gmail.com

without growth regulators. It is the advantage of using these roots for a synthesis of secondary metabolites, which have a practical interest (Kuzovkina and Schneider, 2006).

It is known that the "hairy" root extracts exhibit antimicrobial activity. But the content of biologically active compounds, that contribute to antimicrobial properties, can change dramatically as a result of the bacterial transformation of plants. It was previously shown that transgenic roots of *Ruta graveolens* possess higher antimicrobial activity than control plants (Matvieieva et al., 2015), but the question of spectrum antimicrobial activity of «hairy» roots extracts is still relevant. The activity of *Ruta graveolens* plant extracts, regenerated from "hairy" roots is still unexplored.

The aim of the study was to evaluate the effect of genetic transformation on antibacterial activity of ethanol and water extracts of "hairy" root culture and regenerated plant of *Ruta graveolens* against human pathogenic bacteria and some strains of soil microorganisms.

Material and methodology

Ruta graveolens "hairy" root culture and regenerated from these roots transgenic plants were obtained earlier (Matvieieva and Shakhovsky, 2017). The samples were cultured in *in vitro* conditions on Murashige and Skoog (MS) solidified medium (Murashige and Skoog, 1962) with a twice reduced content of macrosalts. Plants or roots were collected from the nutrient medium, washed with distilled water for obtaining of the extracts. The samples were homogenized with distilled water or 70% ethanol. Then homogenized mass was separated by centrifugation (14 000 rpm for 6 min) in Eppendorf centrifuge. Supernatants were collected and centrifuged again for 4 minutes. The supernatant was applied to discs, the total volume of extract content on each disk was 40 µl.

For these assays, the following microorganisms were used pathogenic test-microorganisms: *Staphylococcus aureus* B-904, *Bacillus subtilis* sub sp. *subtilis*, *Escherichia coli* B-906, *Pseudomonas aeruginosa* B-907; soil bacteria: *Micrococcus luteus*, *Enterobacter hormaechei*, *Kocuria carniphila*, *Citrobacter freundii*, *Rhodococcus erythropolis* and yeast: *Saccharomyces cerevisiae*, *Candida tropicalis* (from the collection of microorganisms of Zabolotny Institute of Microbiology and Virology, NAS of Ukraine). Disco-diffusion method was used for the testing of antimicrobial activity. As a control, we used discs with antibiotics (penicillin, tetracycline, oleandomycin, streptomycin, lincomycin, ciprofloxacin, erythromycin, rifampicin, furagin) and extragents (distilled water or 70% ethanol).

Antibacterial activities were evaluated by the diameter of an inhibition zone (mm) at the end of the incubation time (1–7 days at 25–37 °C in depending on the strain of microorganisms).

Results and discussion

The antimicrobial activities of extracts of the control, transgenic R. graveolens plants, and "hairy" root culture was evaluated (Figure 1).

The results showed that water and ethanol extracts differed significantly in antimicrobial activity.

All kinds of extracts were ineffective against gram-negative bacteria (*Escherichia coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa*) and yeast *Saccharomyces cerevisiae* and *Candida tropicalis* (Table 1).

Water extracts from the control plants demonstrated antimicrobial activity against *Micrococcus luteus*, *Enterobacter hormaechei*, *Kocuria carniphila*. Ethanol extracts from the control plants inhibited growth of *Micrococcus luteus*, *Enterobacter hormaechei*, *Kocuria carniphila*, *Rhodococcus erythropolis* and *Staphylococcus aureus* bacterial strains. Ethanol extracts from the control plants showed twice higher antimicrobial activity against *Enterobacter hormaechei*, because inhibition zones were 12 and

24 mm in the case of using of water and ethanol extracts, respectively. The same time there was some difference in antimicrobial activity of water and ethanol extracts against *Kocuria carniphila*. Ethanol extracts of the control plants have shown five times more active against *Micrococcus luteus* than water extracts.

It was shown that extracts of transgenic roots inhibited growth of *Micrococcus luteus*, *Staphylococcus aureus*, *Enterobacter hormaechei*, *Kocuria carniphila*, *Rhodococcus erythropolis*. In addition, ethanolic extracts inhibited grows of these bacteria and also grows of *Bacillus subtilis*. Ethanol extracts had two times higher antimicrobial activity against all of these microorganisms, except *Staphylococcus aureus* and *Bacillus subtilis* (Figure 1).

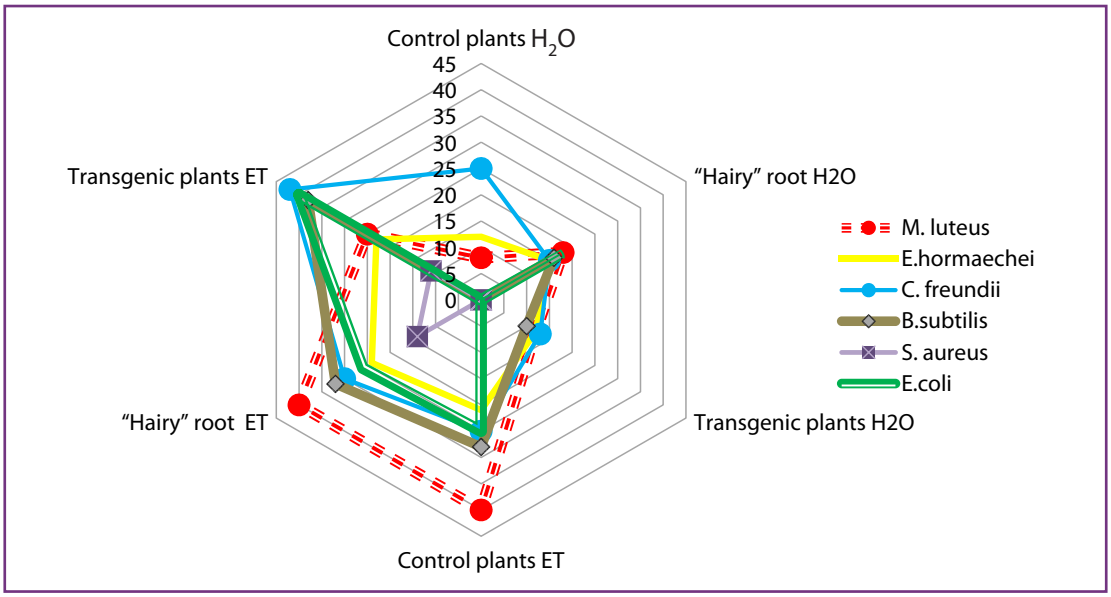

Figure 1 Zones of inhibition of microorganisms growth depending on the type of extract (water and ethanol) of *Ruta graveolens* L. control plants, transgenic plants, and "hairy" root culture

We did not find the significant difference in antimicrobial activity in an extract of "hairy" root culture and transgenic plants with the exception of the effect of the water extract on *Staphylococcus aureus*. It must be noted that only ethanol extract of transgenic *Ruta graveolens* plants inhibited a growth of *Staphylococcus aureus*.

One of the practical problems is a study of changes of antimicrobial activity after *Agrobacterium*-mediated transformation of plants. It was shown that in some cases the extracts from the transgenic plants demonstrated antimicrobial activity nonrelevant for the mother plants. For example, water extracts from the control plants were not active against *Rhodococcus erythropolis*, but water extracts from transgenic plants inhibited a growth of these bacteria. In addition, ethanol extract from the transgenic plants demonstrated greater antimicrobial activity against *Rhodococcus erythropolis* than the extract from the control plants.

Thus as it shown at Figure 1, the ethanol extracts demonstrated higher antimicrobial activity than the water one. This kind of extracts from "hairy" root culture and transgenic *Ruta graveolens* plants more effectively inhibited grows of microorganisms than ethanol extracts from the control plants.

Table 1 The diameter of the inhibition zones (mm) of microorganisms growth by extracts of *Ruta graveolens* L.

Strain	Extracts of *Ruta graveolens*					
	control plants		"hairy" roots		transgenic plants	
	H_2O*	ET**	H_2O	ET	H_2O	ET
Micrococcus luteus	8	40	18	40	12	25
Enterobacter hormaechei	12	21	15	24	12	23
Candida tropicalis	0	0	0	0	0	0
Kocuria carniphila	25	25	15	30	13	42
Citrobacter freundii	0	0	0	0	0	0
Rhodococcus erythropolis	0	28	16	32	10	38
Bacillus subtilis	0	0	0	14	0	11
Staphylococcus aureus	0	25	17	26	0	40
Escherichia coli	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	0
Saccharomyces cerevisiae	0	0	0	0	0	0

* water extract; ** ethanol extract

As the control, we used discs with antibiotics. The studied microorganisms were mostly sensitive to such antibiotics as Ciprofloxacin, Rifampicin, Furagin (Figure 2).

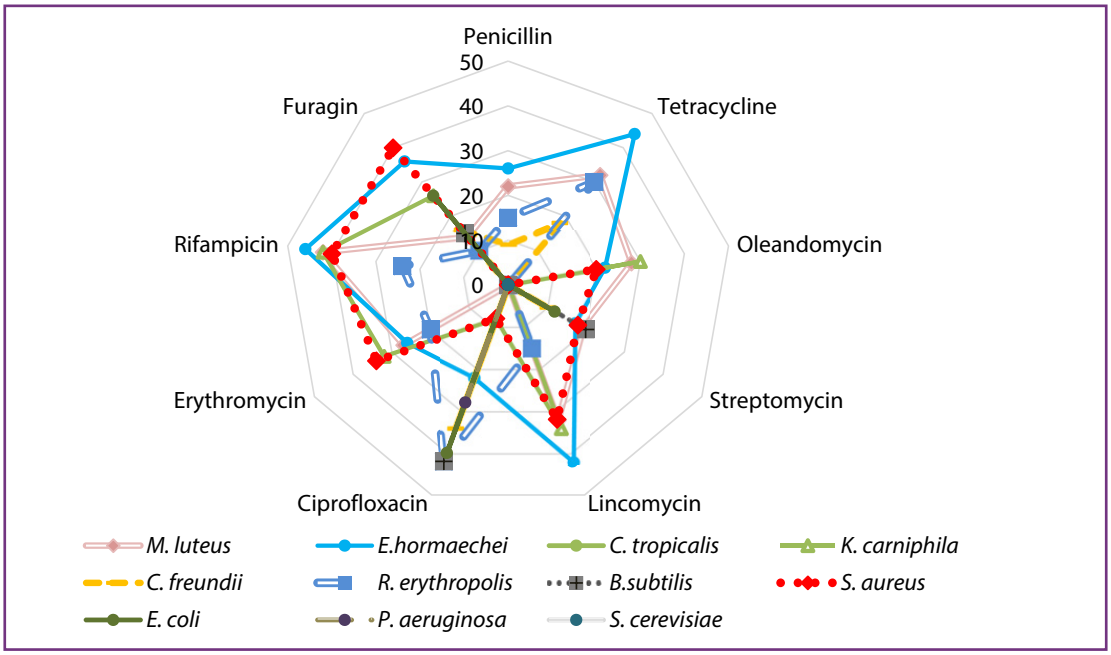

Figure 2 Zones of inhibition of microorganisms growth depending on using of different antibiotics

Conslusion

It was shown that genetic transformation has led to the changes in *Ruta graveolens* antimicrobial activity. These effects resulted in an increasing of the activity against some Gram+microorganisms. At the same time, both water and ethanol extracts from transgenic roots and plants and the extracts from the control plants did not inhibit grows of Gram-negative microorganisms and yeasts.

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