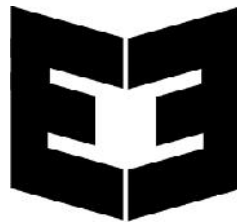


“TERRITORIAL ORGANIZATION OF
SCIENTIFIC AND TECHNICAL UNIONS
AND HOUSE OF SCIENCE AND
TECHNIQUE –
PLOVDIV”

INSTITUTE OF MICROBIOLOGY „STEFAN
ANGELOV” - BAS

NATIONAL SOCIETY OF ECOLOGICAL
ENGINEERING AND ENVIRONMENT
PROTECTION



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ANTIHERPESVIRAL ACTIVITY AND REDOX MODULATING CAPACITY OF WASTE WATER FROM DISTILLATION OF BULGARIAN OILBEARING ROSES

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The aqueous fractions obtained after steam distillation of rose oil are treated as waste products and there is no practice to seek its useful application. However, these waters are rich in water-soluble non-volatile components, mainly polyphenols, and are considered bio-pollutants because they are discharged directly into the soil, drainage system or rivers.

The aim of the present study was to determine the total polyphenol content, redox-modulating capacity and antiviral activity *in vitro* against the replication of herpes simplex virus type-1 in wastewater obtained by distillation of essential oil from the Bulgarian *Rosa alba* L., *Rosa damascena* Mill., *Rosa gallica* L. and *Rosa centifolia* L.

Results: Determination of the total content of polyphenols (galic acid equivalents) by Folin-Ciocalteu method showed that wastewater from *Rosa centifolia* L. is richest in polyphenols, followed by *Rosa gallica* L. and *Rosa alba* L. As DPPH radical scavengers are the most effective wastewater from *Rosa damascena* Mill. at a concentration of 0.01%, the acceptance of DPPH is maximum - 96%. The remaining products showed similar antiradical activity at the tested concentrations. The redox-modulating capacity of the samples (TPTZ test) is as follows: *Rosa alba* L. and *Rosa centifolia* L. > *Rosa gallica* L. > *Rosa damascena* Mill.

The four products tested showed low and relatively close cytotoxicity to MDBK cell cultures. The lowest cytotoxicity is wastewater from *Rosa alba* L., and the highest - those of *Rosa centifolia* L. The maximum tolerance concentration (MTC) of all four products is the same (0.1%). All of them inhibited the replication of the herpes simplex virus type 1 (Viktoria strain HSV -1) - most significantly *Rosa gallica* L. (SI = 12.5), *Rosa centifolia* L. (SI = 9.3) and least *Rosa damascena* Mill.

In conclusion, we would summarize that the wastewater studied by us can serve as a promising resource - a source of polyphenol antioxidants and candidates for possible adjuvants in the prevention and treatment of herpes infection.

Agnowelegment: *The team expresses heartfelt thanks to the financial support of Project of Bulgarian National Sciences Fund -06 36/17 (granted to Assoc. prof. M. Mileva, PhD)*

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3 “ - ”49, Bulgaria , , 6100, .

in vitro

-1 ,

Rosa alba L., *Rosa damascena* Mill., *Rosa gallica* L. *Rosa*

scentifolia L.

:

) Folin-Ciocalteu , (*Rosa centifolia* L.

- , *Rosa gallica* L. *Rosa alba* L.

DPPH - *Rosa damascena* Mill.

0,01% DPPH - 96%.

(TPTZ) : *Rosa alba* L. *Rosa*

centifolia L.> *Rosa gallica* L.> *Rosa damascena* Mill.

MDBK - - *Rosa centifolia* L. *Rosa*

() (0.1%).

1 (Viktoria strain HSV -1) - - *Rosa gallica* L. (SI

=12.5), *Rosa centifolia* L. (SI =9.3) - *Rosa damascena* Mill.

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ANTIHERPESVIRAL ACTIVITY OF ESSENTIAL OILS OF ROSA ALBA L. AND ROSA DAMASCENA MILL. IN VITRO

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ANNA DOBREVA³

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² Institute of Neurobiology, Bulgarian Academy of Sciences, 23, Acad. G. Bonchev, str., 1113 Sofia, Bulgaria

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Rosa alba L., rose with white flowers, belongs to the Rosaceae family, and is commonly known as Bulgarian white rose. *Rosa damascena* Mill. has a long history in Bulgarian folk medicine, folklore, holy beliefs, wedding rituals, and religious traditions. For hundreds of years, both of the roses were grown and processed jointly in the Roses Valley in Bulgaria. Rose oil contains several classes of compounds with different biological activities.

The aim of our study was to determine the redox-modulating capacity and antiviral activity against herpes simplex virus type -1 of *Rosa alba* L. and *Rosa damascena* Mill. oils in infected MDBK cells.

Our results showed that the oils of *Rosa alba* L. and *Rosa damascena* Mill. have good antioxidant activity in a chemical model system: they are good superoxide radical scavenger; they have good reduction capacity with respect to DPPH with a slight predominance of *Rosa damascena* Mill oil; the iron chelation capacity of *Rosa alba* L. oil is close to that of *Rosa damascena* Mill. *Rosa alba* L. oil has a lower toxicity than *Rosa damascena* Mill oil relative to MDBK cells. Pre-treatment of MDBK cells with essential oils protects cells against HSV-1 injuries. *Rosa alba* L. oil shows a significantly stronger effect than *Rosa damascena* Mill oil. Both oils have a positive effect on the state of oxidative stress induced by HSV-1 in MDBK cells. The results were demonstrated by the degree of normalization of lipid peroxidation (TBA test), total glutathione levels, and catalase activity to values close to those of control-uninfected cells after 60 minutes of preincubation.

The results of this pilot study give us reason to assume that the oils of *Rosa alba* L. and *Rosa damascena* Mill. may find application as antioxidant supplements in the comedication of HSV-1 infection, showing good potential for reducing oxidative damage to infected cells.

Agnowelegment: *The team expresses heartfelt thanks to the financial support of Project of Bulgarian National Sciences Fund -06 - 36/17 (granted to Assoc. prof. M. Mileva, PhD)*

ROSA ALBA L. ROSA DAMASCENA MILL. IN VITRO

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Rosa alba L., *Rosa damascena* Mill. Rosaceae

-1 (HSV-1)
MDBK
Rosa alba L. *Rosa damascena* Mill.
:
DPPH
Rosa alba L. *Rosa damascena* Mill.;
Rosa alba L. *Rosa damascena* Mill. Fe (II) -
Rosa damascena Mill. MDBK .
MDBK
HSV-1. *Rosa alba* L.
- *Rosa damascena* Mill.
HSV-1 MDBK

(),
60

Rosa alba L. *Rosa damascena* Mill.

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1,

1,
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2,
1

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Bulgaria

Rosa alba L., *Rosa damascena* Mill., *Rosa gallica* L. *Rosa scentifolia* L.

[CCL-1 (), HaCaT

()], HEK-293 ([HEPG2 ()], A-375 () A-431 ()]
ISO 10993-5/2009,

(/) . *Coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 *C. albicans* SAIMC 562, ISO 20776-1:2006 (E), ().

:

, 50 (IC₅₀) 2,94-8,22, .
: 5.02-12.85 µg/ml . (SI,
IC₅₀ IC₅₀
) 1.46. *R. centifolia* L. - SI 1,9.
IC₅₀ 3.44 µg/ml *R. centifolia* (=
1,95 mg/ml), ()
55%. = 3,6 - 3,85
mg/ml 70%.

Rosa centifolia L.

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-06 36/17 (- . - .)

CYTOTOXICITY AND ANTIMICROBIAL EFFECT OF WASTEWATER OBTAINED FROM DISTILLATION OF BULGARIAN OIL - BEARING ROSES

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The wastewater from the distillation of oil roses is discharged directly into the soil without looking for a way to utilize them. Due to their rich polyphenol composition, they could serve as a raw material with valuable biological properties.

The aim of the present pilot study was to obtain initial data on cytotoxicity on a panel of non-tumorigenic and tumor cell lines, as well as the antimicrobial effect against several opportunistic strains with the cutaneous colonizing potential, of wastewater from the distillation of Bulgarian *Rosa alba* L., *Rosa damascena* Mill., *Rosa gallica* L. *Rosa centifolia* L.

Materials and methods: The cytotoxic potential was tested against three non-tumorigenic [CCL-1 (transformed mouse fibroblasts), HaCaT (transformed keratinocytes), HEK-293 (transformed embryonic kidney cells)] and three tumor cell lines [HEPG2 (hepatocellular carcinoma 1st stage, so it also has properties of a normal cell line), A-375 (malignant melanoma) and A-431 (non-melanoma epidermoid squamous cell skin carcinoma)] according to ISO 10993-5/2009, Annex C. The minimal inhibitory and bactericidal concentrations (MIC/MBC) of wastewater against *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *C. albicans* SAIMC 562 were determined, according ISO 20776-1:2006(E), including the microbial respiratory activity – by MTT test.

Results: The four products tested showed cytotoxicity to all cell lines, but the effect on non-tumorigenic lines was less pronounced. The inhibitory concentration 50 (IC₅₀) in tumor lines is 2.94-8.22, and in non-tumorigenic lines – 5.02-12.85 µg/ml polyphenols. The selectivity index (SI, the ratio of the mean IC₅₀ in non-tumorigenic and the mean IC₅₀ in tumor lines) is 1.46. Wastewater from *R. centifolia* L. has the strongest antitumor effect with an average IC₅₀ in tumor lines of 3.44 µg/ml polyphenols and SI 1.9.

The highest antistaphylococcal effect showed the wastewater from *R. centifolia* (MIC = 1.95 mg/ml phenols), but the dehydrogenase activity (DEHA) was reduced with about 55%. The other three types of wastewater showed antimicrobial effect at MICs = 3.6 – 3.85 mg/ml phenols and decreased the DEHA with about 70%.

Conclusion: The obtained results give us reason to concentrate our efforts on further studies related to a more in-depth elucidation of the mechanisms of cytotoxicity, as well as the inhibition of staphylococcal biofilms in the waste product models used and the most promising in both aspects is *Rosa centifolia* L.

Agnowelegment: *The team expresses heartfelt thanks to the financial support of Project of Bulgarian National Sciences Fund -06- 36/17 (granted to Assoc. prof. M. Mileva, PhD)*

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Rosa

damascena Mill. *Rosa alba* L.,

2020 . , *Rosa centifolia* L.

Rosa damascena *Rosa gallica* L.

0,015

- 0,048 %.

: (17,60 - 34,02 %), (8,38 - 28,7 %) (4,8 -

11,90 %),

%),

(4,37 - 10,21 %),

(1,07 - 2,98 %)

: (8,10 - 22,67

(0,81 - 5,90 %).

70 %,

:

-06- 36/17 (- . - .)

RECENT RESULTS OF THE YIELD AND COMPOSITION OF ROSE OIL FROM THE OIL-BEARING ROSES, CULTIVATED IN BULGARIA

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Bulgaria is famous for its oil-bearing rose. *Rosa damascena* Mill and *Rosa alba* L. are mainly cultivated in the country, but a recent survey of industrial plantations in 2020 revealed that *Rosa centifolia* L. and hybrids of *Rosa damascena* Mill. x *Rosa gallica* L. are also common in the rose valley. Although their essential oil can not be compared in quality with the classic, they are preferred by farmers with high yields of flowers and resistance to diseases and pests. They are used to produce rose water and extracts.

Our study showed that the yield of essential oil from these roses is in the range of 0.015 - 0.048%. The main group in the chemical composition are terpene alcohols, which vary in the range: geraniol (17.60 - 34.02%), citronellol (8.38 - 28.7%) and nerol (4.8 - 11.90%), but with a different ratio. Hydrocarbons are represented by saturated aliphatic homologues with an odd number of carbon atoms, the main ones being: nonadecane (8.10 - 22.67%), heneicosane (4.37 - 10.21%), heptadecane (1.07 - 2, 98%) and tricosane (0.81 - 5.90%).

Interestingly, in mixed plantations with predominance of damascene over 70%, the composition retains the parameters of its oil and the presence of other genotypes should be proven by special analysis techniques.

Agnowelegment: *The team expresses heartfelt thanks to the financial support of Project of Bulgarian National Sciences Fund -06- 36/17 (granted to Assoc. prof. M. Mileva, PhD)*