7th Workshop

SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

September 22nd 2021, Vinča Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

PROCEEDINGS

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ANTIBACTERIAL ACTIVITY OF AQUEOUS-ETHANOLIC EXTRACTS OF *Alchemilla vulgaris* AND *Frangula alnus* COMBINED WITH STREPTOMYCIN

S. Đukanović[1], S. Cvetković[1], T. Ganić[1], B. Nikolić[1], N. Tomić[2], D. Kekić[3] and D. Mitić-Ćulafić[1]

[1]University of Belgrade, Faculty of Biology, Studentski trg 12-16 11000 Belgrade, Serbia (stefana.d@bio.bg.ac.rs)
[2]Institute of Technical Sciences of SASA, KnezaMihaila 35/IV,11000 Belgrade, Serbia
[3]University of Belgrade, Faculty of Medicine, Microbiology Institute, Dr Subotića 1, 11000 Belgrade, Serbia

ABSTRACT

The harmful effects that antibiotics may have on human health and increased resistance of microorganisms on antibiotics made pharmaceutical companies look for new alternatives among medicinal and aromatic plants. Recently, as a new strategy to enhance antimicrobial activities of commercial antibiotics, the combination of plant extracts and antibiotics is developing. The aim of the study was to examine the potential synergistic effect of plant extracts with streptomycin. The minimum inhibitory concentration of two extracts obtained from traditional medicinal plants, *Alchemilla vulgaris* and *Frangula alnus*, was determined by the microdilution method. The effect of a mixture of extracts and antibiotics was tested with checkerboard assay and confirmed by Time kill assay. The most sensitive strain was *E. coli* ATCC 25922.

INTRODUCTION

In recent years, we are facing a serious problem of bacterial resistance. The development of new antibiotics and antibacterial compounds cannot follow the spread of resistance. In order to solve this problem, it is necessary to find new antibacterial compounds from different sources, primarily from natural ones. Plants are rich in various secondary metabolites that have numerous biological activities, including antibacterial [1]. The use of plants in traditional medicine makes them one of the important sources of potential antimicrobial compounds. The combination of plant extracts and commercial antibiotics is one of the ways to slow down the spreading of resistance [2]. Combined use would reduce the amount of antibiotics and, on the other hand, increase its efficacy at lower concentrations. Additionally, it would reduce the period of treatment. *Alchemilla vulgaris* and *Frangula alnus* are widely used in folk

medicine. *A.vulgaris*, a long-standing herbaceous plant from the *Rosaceae* family, has application in the treatment of wounds, eczema, digestive problems and gynaecological disorders [3]. *F. alnus* grows like a bush or low tree and belongs to the family *Rhamnaceae*. As a medicinal drug, the bark of the plant is used. It is taken internally as a laxative and is also used to treat abdominal bloating, hepatitis, cirrhosis, liver and gall bladder complaints [4].

The aim of the study was to test the antibacterial activity of aqueous-ethanolic extracts of *A. vulgaris* and *F. alnus* and to determine the type of interaction of extracts and streptomycin.

EXPERIMENTAL

The minimum inhibitory concentration (MIC) was determined by the microdilution method with adding resazurin as a cell viability indicator. The concentration of extracts was tested in a range of 2 mg/mL-0.015 mg/mL, and streptomycin was used as a positive control. From 10 tested strains, only *S. aureus* MRSA ATCC 33591, *P. mirabilis* ATCC 29906, and *E. coli* ATCC 25922 were sensitive to extract. Because of that, they have been selected for further study. To determine the type of interaction between extracts and streptomycin checkerboard assay was applied. In the microtitar plate, a double gradient of extracts (vertically) and streptomycin (horizontally) was made. The results of combined treatment were evaluated by calculation of fraction inhibitory concentration index (FICI) according to the formula: FICI = (MICA comb / MIC A alone) + (MIC B comb / MIC B alone). Due to FICI, the effect of combination can be synergistic (FICI $\leq$ 0.5), additive (0.5< FICI $\leq$ 1), indifferent (1< FICI $\leq$ 4) and antagonistic (FICI > 4). Concentrations in combination that showed synergistic effect were tested by Time kill assay for the results verification. The samples were inoculated with 10^5 CFU/mL in the following order: medium and bacteria (control), medium and extract, medium and streptomycin and medium with the mixture (extract+streptomycin). Samples were incubated at 37˚C for 24h. Sampling was done periodically after 0, 1, 2, 4, 6, 8, and 24 hours of incubation. CFU/mL was determined after the application of appropriate dilution on the LA substrate and incubated for 24 hours at 37˚C.

RESULTS AND DISCUSSION

Microdilution assay on 10 tester strains was performed with extracts that were applied in a concentration range of 2 mg/mL-0.015 mg/mL. Obtained results showed that only *S. aureus* MRSA ATCC 33591, *P. mirabilis* ATCC 29906, and *E. coli* ATCC 25922 were sensitive to any extracts. Determined MIC values are presented in Table 1. *F. alnus* exhibited moderate antibacterial activity against *S. aureus*, while Sadowska et al. [4] found strong antistaphylococcal activity (MIC=0.75 mg/mL). Also, the antibacterial effect of *A. vulgaris* was detected on *P. mirabilis* and *E. coli* ATCC 25922 and was more

pronounced on *P. mirabilis*; similar results were obtained by Boroja et al. [3]. Slight discrepancies between the results obtained in this study and literature data could be explained by differently prepared extracts.

Due to observed sensitivity, these strains have been selected for further study, searching for types of interaction between extracts and streptomycin.

Table 1. Minimum inhibitory concentrations of extracts and streptomycin.

	F.alnus	***A.vulgaris***	**Streptomycin**
S.aureus MRSA ATCC 33591	2 mg/mL	> 2 mg/mL	6.25 µg/mL
P.mirabilis ATCC 29906	> 2 mg/mL	1 mg/mL	25 µg/mL
E.coli ATCC 25922	> 2 mg/mL	2 mg/mL	12.5 µg/mL

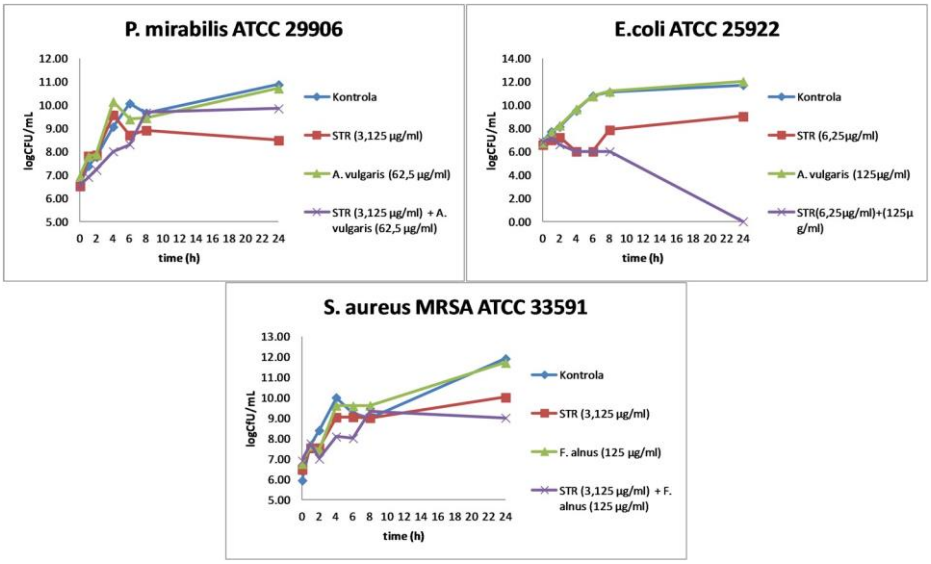

Figure 1. Synergistic effect of extracts and streptomycin.

Furthermore, the synergistic effect was analyzed once again by determining the generation time from the log phase of growth. The time needed for bacteria to double their number is prolonged when the co-treatment was applied (Table 2).

Results obtained by checkerboard assay showed that concentrations of extracts tested in a range of 62.5 µg/mL-250 µg/mL and streptomycin in the range of 1.625 µg/mL-6.25 µg/mL exhibited a synergistic effect only on *E. coli* ATCC 25922 (Figure 1). Both extracts led to an increase in the activity of streptomycin on *S. aureus* MRSA ATCC 33591 and *P. mirabilis* ATCC 29906, and we assume that it could be a bacteriostatic effect. According to Mulyaningsih et al., [5], the confirmation of the existence of synergistic effect is verified after 24h of treatment, as was observed in *E. coli* ATCC 25922 in the Time kill assay.

Table 2. The generation time of tested bacterial strains.

	Control	**Streptomycin**	**Extracts**	**Combination**
S.aureus MRSA ATCC 33591	25min	20min	15min	140min
P.mirabilis ATCC 29906	25min	20min	20min	120min
E.coli ATCC 25922	25min	25min	25min	45min

CONCLUSION

Extracts showed antibacterial activity, especially in combination, on several bacteria that are resistant to many antibiotics. The synergistic effect was obtained only with the combination of *A. vulgaris* extract and streptomycin on *E. coli* ATCC 25922 after 24h of incubation. Due to the observed synergy with streptomycin, both extracts may be recommended for further research as good candidates for use as dietary supplements.

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REFERENCES

[1] F. Bourgaud, A. Gravot, S. Milesi, E. Gontier, Plant Science, 2001, 161, 839–851

[2] M.El-Azizi, International Journal of Microbiology, 2016, 2016, 10

[3] T. Boroja, V. Mihailović, J. Katanić , S.-P. Pan, S. Nikles, P. Imbimbo, D.M. Monti, N. Stanković, M.S. Stanković, R. Bauer, South African Journal of Botany, 2018, 116, 175–184.

[4] B. Sadowska, M. Paszkiewicz, A. Podsędek, M. Redzynia, B. Różalska, 2014, ActaBiochimicaPolonica, 61, 163-169..

[5] Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J., Wink, M., 2010. Phytomedicine 17, 1061-1066.

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