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ANTIBACTERIAL ACTIVITY OF *VERBASCUM SONGARICUM* VARIOUS EXTRACTS AGAINST *STAPHYLOCOCCUS*

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Abstract

Over the last 15 years, Staphylococcus aureus is one of the leading causative agents of post-operative and posttraumatic purulent wound complications in traumatology and orthopedics, such as osteomyelitis, abscess, cellulitis. Due to the frequent occurrence of multiple antibiotic resistance of Staphylococcus aureus is necessary to search for new high-performance antibacterial drugs. Verbascum songaricum Schrenk, a member of a genus Verbascum (Scrophulariaceae), is a biennial plant that traditionally used in folk medicine. Verbascum species reported to have expectorant, mucolytic and demulcent properties, and are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma in traditional medicine of different nations. They also used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea. The oil from the flowers of mullein is used to help soothe an earache and can be applied externally for eczema and other types of inflammatory skin conditions.

Therefore, the aim of this study was to determine the antibacterial effects of the methanol, water, chloroform, ethyl acetate and hexane extracts of V. songaricum were investigated for their antimicrobial activity against microbial strains of Staphylococcus aureus using microdilution method. Water extract showed the highest activity against Staphylococcus aureus (growth=4.5837%), which is comparable with the activity of standard antibiotic (growth=2.4397). Also, Staphylococcus aureus was susceptible to the methanol extract of V. songaricum, the inhibition was 85.1770%.

In conclusion, Verbascum songaricum water and methanol extracts demonstrating antibacterial activity against Staphylococcus aureus could result in the discovery of novel antibacterial agents; this may help to discover new antibiotics that could serve as selective agents against infectious diseases.

Keywords: *Verbascum songaricum, extracts, antimicrobial activity, Staphylococcus aureus, microdilution method.*

Introduction

In a series of clinical observations and laboratory researches published in 1880 and 1882, Ogston described staphylococcal disease and its role in sepsis and abscess formation [*Ogston A, 1882; Ogston A, 1884]*. Nowadays, more than 100 years later, Staphylococcus aureus remains a versatile and dangerous pathogen in humans. The frequencies of both community-acquired and hospital-acquired staphylococcal infections have increased steadily *[Brumfitt W, Hamilton-Miller J, 1989*]. Over the last 15 years, *Staphylococcus aureus* is one of the leading causative agents of postoperative and posttraumatic purulent wound complications in traumatology and orthopedics, such as osteomyelitis, abscess, cellulitis. Due to the frequent occurrence of multiple antibiotic resistance of *Staphylococcus aureus* is necessary to search for new high-performance antibacterial drugs [*Belobrodov V, 2003*].

Verbascum songaricum (*Scrophulariaceae*),

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Verbascum L., common name Mulleins, is a genus of 360 species of flowering plants, predominantly distributed in Asia, Europe, and North America. Since ancient times, Mullein has been used as a medicinal herb. The leaves and flowers are reported to have expectorant and demulcent properties, which are used to treat respiratory problems such as bronchitis, dry coughs, whooping cough, tuberculosis, asthma, and hoarseness. *Verbascum* species are also used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea, and have inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B. The oil from the flowers of mullein is used to help soothe an earache and can be applied externally for eczema and other types of inflammatory skin conditions [*Tatli I et al., 2004*].

Verbascum songaricum Schrenk that is the subject of the current study and one of the growth areas of the species in the foothills of Dzhungar and Tien Shan mountains. Although V. songaricum herb is used among the local people for the purpose of remedy and it is widespread in Kazakhstan, the information on V. songaricum is relatively limited. It has been studied in details regarding its saponin content in aerial parts and flavonoids in the roots, while the presence of iridoids has been proven only by TLC [*Seifert K, 1991; Hartleb I, Seifert K, 1994; Hartleb I, Seifert K, 1995; Yuldashev M, 1996; Grabias B, Swiatek L, 1987*]. The content of fatty acids was established using gas chromatography method [*Makhatova B et al., 2015*]. The goal of our work was to determine the antimicrobial activity of *Verbascum songaricum* extracts against *Staphylococcus aureus*.

Experimental part

Plant material; Aerial parts of V. songaricum Schrenk were collected from Tulkubas region, Kazakhstan during the months of May-June 2014.

Preparation of extracts: The plant parts were air-dried and then dry powdered plant material extracted with methanol (Sigma-Aldrich, Germany) using maceration extraction method. The extract was filtered with filter paper and the filtrate was evaporated under vacuum in a rotary evaporator at 40°. When using the method of liquid extraction hexane, chloroform, ethylacetate and water extracts obtained. The obtained hexane, chloroform and ethyl acetate extracts evaporated to dryness under vacuum in a rotary evaporator. The aqueous extract evaporated in the lyophilic dryer. The dry extracts were stored in labelled sterile screw-capped bottles at - 20° pending use.

Antimicrobial activity – microdilution assay: Reference microbial strains of *Staphylococcus aureus* ATTC 29213 were obtained from the laboratory of Department of Infectious Diseases and Microbiology (Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno). Tested extracts dissolved in dimethyl sulfoxide (DMSO) were serially diluted using cultivation medium and transferred in quadruplicates to 96-well flat-bottom microplates.

The fresh overnight culture of *S. aureus* was resuspended in Mueller Hinton Broth to afford final target inoculum of 6×10^5 CFU/mL in a well. Tested extracts were added to the 100 µL of yeasts suspension in the 96-well plate to obtain the final concentrations of 512, 256, 128, 64 µg/mL and the growth was continuously monitored by measuring of the absorbance at 600 nm in the microplate reader (BMG Reader Labtech, Germany) at 37°C for 0 to 24 h. Bactericide Ciprofloxacin (1 µg/mL) was included as a positive control.

Results and discussions

Verbascum L. species contain biologically active compounds such as flavonoids, phenyl ethanoid and neolignan glycosides, saponins, and iridoid and monoterpene glycosides. There are many investigations on antimicrobial activities of various Verbascum species. The methanol extracts obtained from *V. gypsicola Vural* and *Aydogdu, V. pseudoholotrichum Hub-Mor., V. cymigerum Hub-Mor., V. cholorostegium Bornm* and *Murb, V. linguifolium Hub-Mor., V. pellitum Hub-Mor., V. dalamicum Hub-Mor., V. chionophyllum Hub-Mor., V. cilicium Boiss, V. trapifolium (Stapf) Hub-Mor., V. meinckeanum Murb* and *V. lyratifolium Kochel* were studied for their antimicrobial activities against *E. coli, S. aureus, K. pneumonia, P. aeruginosa, B. cereus, M. smegmatis, L. monocytogenes, M. luteus, C. albicans, R. rubra* and *K. fragilis* by disk diffusion method [*Makhatova B et al., 2016*]. The *Verbascum L.* extracts had a strong antimicrobial activity against the Gram-positive bacteria in the yeast cultures used in these studies. In another study, the methanol extracts of the flowers,

leaves, seeds and roots of *V. phlomoides, V. blattaria, V. nigrum, V. bombyciferum, V. chaixii, V. dumulosum, V. olympicum, V. phoeniceum* and *V. roripifolium* were studied for their antimicrobial activity against *E. coli, P. aeruginosa, S. aureus* and *C. albicans*. It was determined that antimicrobial activity of the methanolic extract of *V. sinuatum* showed inhibition against all the bacterial strains tested (MIC between 15.5 and 250 μg/mL).

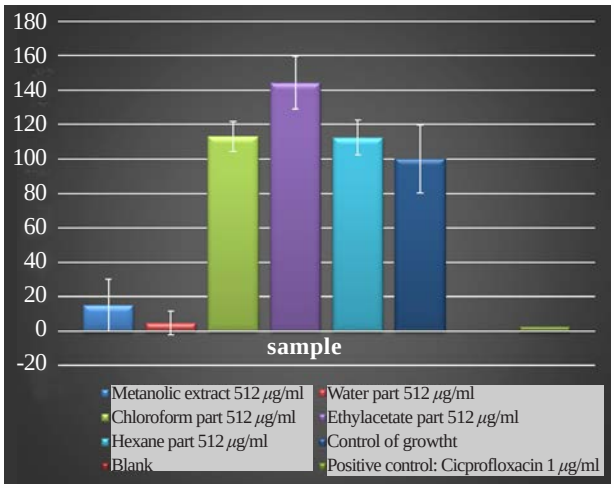

***Figure1** - Growth of Staphylococcus aureus for 120 hours %.*

In this study, the methanol, water, chloroform, ethyl acetate and hexane extracts of *V. songaricum Schrenk* aerial part were tested by microdilution assay for their antimicrobial activity against *Staphylococcus aureus* (Fig.1).

Chloroform, ethyl acetate and hexane extracts have not antimicrobial effect. Water extract showed the highest activity against *Staphylococcus aureus* (growth = 4.5837%), which is comparable with the activity of standard antibiotic (growth=2.4397%). Also, *Staphylococcus aureus* was susceptible to the methanol extract of *V. songaricum*, the inhibition was 85.1770%.

Metanolic extract 512 ug/ml, Chloroform part 512 ug/ml, Hexane part 512 ug/ml, blank, Water part 512 *ug/ml*, Ethyacetate part 512 ug/ml, control of growth, positive control cicprofloxacin 1 ug/ml

Based on the results, it is possible to conclude that water and methanol extracts are better solvents for extraction of antimicrobial substances against *Staphylococcus aureus* from *Verbascum songaricum* herb.

In conclusion, *Verbascum songaricum* water and methanol extracts demonstrating antibacterial activity against *Staphylococcus aureus* could result in the discovery of novel antibacterial agents; this may help to discover new antibiotics that could serve as selective agents against infectious diseases.

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