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Original scientific paper

ANTIBACTERIAL PROPERTIES OF WHITE LILY (*Lilium candidum)* EXTRACT

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Abstract: Natural extracts made from fresh plant material and biologically active compounds isolated from different plant species that have been used in folk medicine for centuries, may present valuable sources for production of novel natural preservatives and medicinal preparations. White lily-*Lilium candidum* is also used, for a long period of time, in folk medicine for healing of burns and frostbites, otitis and rhinitis as well as balm for washing wounds and cuts. Roots, leaves and flowers have medical properties. Nevertheless, there is little information in literature related to antibacterial effect of white lily extract on certain pathogens and there is a need to determine the justification for its use in traditional medicine.

Antibacterial activity of White lily extract on five reference cultures (*Escherichia coli* WDCM 00090, *Listeria monocytogenes* WDCM 00020, *Salmonella enterica* WDCM 00030, *Pseudomonas aeruginosa* WDCM 00024) and seven bacterial cultures (*Escherichia coli, Providencia stuartii, Pseudomonas* spp. ß-hemolytic *Escherichia coli*, coagulase positive staphylococcus, *Staphylococcus aureus*, group D *Sreptococcus*) from Laboratory for Microbiology, Public Veterinary Institute of Republic of Srpska "Dr. Vaso Butozan", Banja Luka, were examined in this study. Results confirmed that white lily extract show certain antibacterial activity against examined pathogens used in this study. Antibacterial activity of White lily extract was in the range from 5.33 mm to 18.88 mm depending of bacterial strain and concentration of white lily extract.

Key words: *Lilium candidum*, antimicrobe activity, White lily extract

INTRODUCTION

Plant usage for medical purposes is widespread and nowadays 30-40% of all medical preparations contain one or more plant isolated bioactive components. More attention is given to usage of natural phitochemical components that own antimicrobial, antioxidative, antifungal, anticarcinogen, antimflammatory and other properties that support prevention and therapy of many current diseases (Fahimi, 2015, Rai, 2013, Brochardt et al., 2008).

The healing properties of White lily is known for long period of time and is very appreciated in folk medicine. Numerous prescriptions from the Elizabethan era testify about usage of Lily`s oil for medical purposes, since it was believed that Lily has magical power and was used in fever healing, as a balm for washing wounds as well as lining in treatment of burns and ulcer, but also for relieving symptoms of arthritis and rheumatism.

White lily (*Lilium candidum)* is a perennial plan that may grow up to 100 cm in high. It has characteristic smell and pretty big white funnel shaped flower with wide and long petals. Because of its beauty and specific smell, it is planted as ornamental plant in gardens and plants (Lesinger, 2016).

Lilium candidum contains different biologically active components which act antimflammatory, antimutagenic, antioxidative, antiviral (Fahimi et al., 2015, Lesinger, 2016, Eisenreichová et al., 2004**,** Mucaji, 2007). Usage of lily`s oil in traditional medicine lasts for centuries, but modern medicine have disputed its medical properties for a long time. Nevertheless, experimental studies have confirmed some medical properties (Wang et al., 2019, Fahimi et al., 2015, Jie et al., 2015, Zhang et al., 2017).

Several studies that examined content and characteristics of *Lilium candidum*, as well as its agenspositive/negative effects on organism, have been obtained during last twenty years (Wang et al., 2019, Fahimi et al., 2015, Zhao et al., 2015, Bates, 2015, Kopaskova et al., 2012, Huang, 2015).

Lily, i.e. its flowers, leaves and bulb, are rich in flavonides, glikosamides, organic acids, nitrogen and steroid compounds, saponines, vitamin C, tanine, eteric oils, while bulb additionally contains bitter matters, holing and phytosterol (Lesinger, 2016, Wang et al., 2019, Eisenreichová et al., 2004)**.** As plant tissues and extracts contain tannin compounds, for which is known to react with skin proteins, by applying the White lily extract on skin, burn, i.e. injured or diseased part, tannin reacts with protein, i.e. collagen fibers and creates a layer that protects tissue from further development of necrosis (Fahimi et al. 2019, Kovačević, 2004).

In contact with cells, tissues and microorganisms, White lily extract exerts an effect i.e. improve/amplify ability of organism to respond to certain physical and chemical impacts in a way to " vacuum" smaller doses of the same or similar agent (Lesinger, 2016, Achary et al., 2012).

White lily extract is obtained by maceration of fresh flowers in unrefined olive oil and is known for its anti-inflammatory effect. It is dissolved well in alcohol and acetone, and not so well in organic solvents (Kopaskova et al., 2012, Eisenreichová et al., 2004). White lily extract is quite used in pharmaceutical-cosmetic industry due to its beneficial effect on skin (improved epitelisation). It is also used for treatment of various rashes, strains, lichens, psoriasis and eczema. It is also used for everyday care of mature and dry skin, skin that needs to be revitalized and is excellent protector of skin during winter days (Lesinger, 2016, Huang et al., 2015).

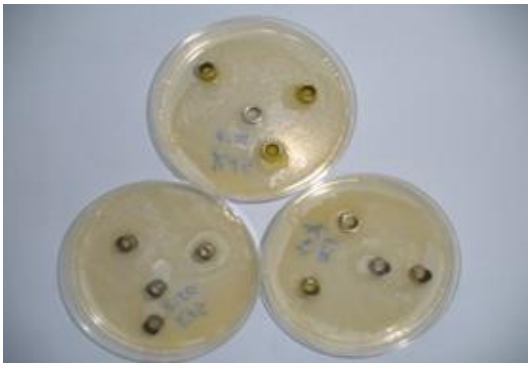
There is little data in available literature related to antibacterial properties of Lily oil extract and due to that the aim of this study is to examine antibacterial properties of Lily oil extract on certain pathogens and to establish justification of its usage in traditional (folk) medicine and to establish weather it has a bactericidal or bacteriostatic action on examined bacterial cultures.

MATERIAL AND METHODS

Fresh flowers with ferns of White lily *Lilium candidum*, sampled from plantation "Klindić", Banja Luka, Republic of Srpska, were used as row material for production of oil extract. Fresh flowers with ferns are immersed in a jar filled with cold pressed olive oil in quantity 1:3. Well closed jar is kept on sunlight for 40 days, with occasional shaking. Prepared and for a while kept oil is filtered and kept in dark bottle protected from direct impact of sunshine. White lily extract is mixed with 96% alcohol in concentrations: 1:2, 1:5 and 1:1

Picture 1. Disk diffusion test

For examination of antibacterial activity of White lily extract reference culture *Escherichia coli* WDCM 00090, *Listeria monocytogenes* WDCM 00020, *Salmonella typhi* WDCM 00030, *Salmonella enterica* WDCM 00030, *Pseudomonas aeruginosa* WDCM 00024 (BCCM™/LMG BACTERIA COLLECTION, Belgium) were used and isolated from culture collection of Laboratory for Microbiology of Veterinary Institute "Dr Vaso Butozan" Banja Luka: *Escherichia coli, Providencia stuartii, Pseudomonas spp.,* ß-hemolytic *Escherichia coli,* coagulase positive staphylococus*, Staphylococcus aureus, Streptococcus group D* (urine, throat swab, nasal swab, humans and animals). Cultures were seeded in nutrition broth and incubated on 37^0C/18h. Petri dishes with adequate media (Müeller - Hinton agar) were seeded with 0.1 ml of bacterial suspension whose concentration was 10^5cfu/ml.



Picture 2. Reading the results

For examination of White lily extract on growth inhibition of selected bacterial species, agar diffusion method was used (Kirby-Bauer, 1996) on solid sterile nutrition broth (Müeller-Hinton agar - MHA). Cylinders with 9 mm diameter were settled on solid culture media

surface which was previously seeded by certain pure bacterial culture. In cylinders, 100 µl of certain quantity (1:2; 1:5 i 1:10) of extract and alchohol was pured, drop by drop by micropipete. To eliminate impact of olive oil on antibacterial effect of White lily extract, as control, drop by drop 100 µl of olive oil was pured in cylinders. Capability of growth and reproduction of strain depends on its sensitivity to examined extract, so clear transparent zone whithout microorganism growth is formed around cylinder if effect exists. Prepared plates were kept on room temperature for 30 minutes in order to provide evenly diffusion in media, and then were incubated on 37°C/24 hours.

For each microorganism and for each concentration of White lily extract three repetitions were obtained, and results were read as diameter of growth inhibition zone and presented as average in millimeters.

In order to see if White lily extract has bactericide or bacteriostatic power, little piece of agar was taken from inhibition zone and added to nutrition broth. Incubation was obtained on 37ºC/24h. If broth was turbid after incubation, it is considered that oil had bacteriostatic effect, i.e., if broth stayed cleared after incubation, oil had bactericide effect.

RESULTS AND DISSCUSION

Antibacterial activity of of White lily extract on five reference and seven bacterial cultures isolated from clinical materials was examined in this study. Obtained results are presented by tables and figures (Table 1. and Figure 1.).

Obtained results indicate that White lily extract oil perform antibacterial activity on all tested bacterial species, and intensity of action differed and ranged from 5.33 to 18.33 mm.

Table 1. Growth inhibition zone of tested bacterial species realized with different concentrations of White lily extract oil and ethanol

Microorganism	White lily extract : ethanol			
	Extract	**1:2**	**1:5**	**1:10**
Salmonella typhi WDCM 00031	12.00±2.65	13.00±6.08	9.33±2.31	14.33±5.51
Salmonella enterica WDCM 00030	11.66±1.53	15.00±0.00	14.66±5.03	16.66±7.23
Escherichia coli WDCM 00090	13.00±1.00	18.33±2.89	16.66±2.89	12.33±3.06
Pseudomonas aeruginosa WDCM 00024	7.00±0.00	10.33±4.04	5.33±4.62	14.33±6.03
Listeria monocytogenes WDCM 00020	11.66±1.53	16.66±5.77	13.33±5.77	8.33±7.64
Pseudomonas spp	7.66±1.15	6.66±5.77	8.66±1.15	12.33±0.58
Escherichia coli	8.66±1.15	6.33±5.51	6.66±5.77	18.33±5.69
ß hemolytic *Escherichia coli*	14.66±0.58	18.00±2.65	18.33±5.77	14.00±1.73
Streptococcus group D	11.00±7.73	10.00±2.00	13.00±2.00	15.66±4.04
Staphylococcus aureus	8.66±1.15	8.00±0.00	11.00±3.61	13.00±2.65
Coagulase positive staphylococcus	7.33±0.58	8.00±0.00	7.66±7.51	9.00±1.00
Providencia stuartii	7.66±0.58	8.00±0.00	8.00±0.00	8.00±0.00

Showed values are given in mm and present average value of inhibition zone for three measurements. White lily extract showed the highest antibacterial activity towards *β* hemolytic *E. coli* with inhibition zone from 14.00 mm to 18.33 mm and something less intensive inhibitory effect towards reference *E. coli* strain with inhibition zone from 12.33 mm to 18.33 mm, while it affected *E. coli* (clinical isolate) mostly in combination with alcohol 1:10 with inhibition zone 18.33 mm. Also, it is important to emphasize that there was stronger antibacterial effect of extract and alcohol in concentration 1:10 on *P. aeruginosa* reference (14.33 mm) and clinical isolate (12.33 mm).

White lily extract showed lowest or the weakest antibacterial activity towards clinical isolates of coagulase positive staphylococcus (inhibition zone 7.33mm to 9.00 mm) and *Providencija stuartii* (7.66 mm to 8.00 mm) in all combinations, that can be explained by different solubility of White lily extract and its components. Hydophobicity is important characteristic of eteric oils and extracts since it increases bacterial cell membrane permeability and provides easier passage of components within its lipid layer. Altered permeability of cell membrane is usually associated with the loss of cell osmotic control, which is considered as fundamental principle of antimicrobial action of extracts and eteric oils (Bajpai et al., 2012, Bubonja et al., 2008). Because of different solubility of antibacterial components in water, different methods of examination of antibacterial activities are used. In this study, disk diffusion method which totally depends on hydrophobicity of the active components and the rate of their diffusion through the agar, was used (Bubonja et al., 2008).

The latest studies in the field of chemistry, biochemistry and medicine confirm that plant extracts contain phenolic acid, flavones, isoflavones, flavanols, catechins, tocopherols, tannins, terpenes ant to show antimicrobial, antineoplastic, antiviral, antiinflammatory, antiallergic and avtioxidant properties (Wang et al., 2019, Al-Bayati, 2018, Jia, 2015, Li et al., 2018, Jin et al., 2014, *Han and Xie*, 2013, Javed et al., 2012, Lu et al., 2013). Phenol components have toxic effect on microorganisms, and mechanism of action includes inhibition of oxidized components, as well as possible reaction with sulfhydryl groups through more non specific reactions with proteins. Polyphenolic compounds are accumulated mainly in cell walls and mostly on yield`s surface (epidermal and subepidermal leyers), since biosynthesis of these compounds is light dependent (Marzio et al., 2011, Pjanović et al. 2010, Rai, 2013).

Results of this study indicate on antibacterial potency of White lily extract and are in accordance with results of other researchers who studied plant extracts (Fahimi et al., 2015, *Patocka and Navratilova,* 2019, Devi et al., 2016, Capasso et al., 2005, Wang et al., 2012, Matejić et al., 2012, Sedighinia, 2012), but also with those who examined chemical composition and antimicrobial effect of Lily eteric oil (Fahimi et al. 2015, Wong et al., 2019, Bates et al., 2015, Achary et al., 2012, Yarmolinsky et al., 2009).

In order to resolve whether White lily extract has bactericide or bacteriostatic power, little piece of agar was taken from inhibition zone and added in nutrition broth.

For better presentation, type of action of White lily extract is presented on Figure 1.

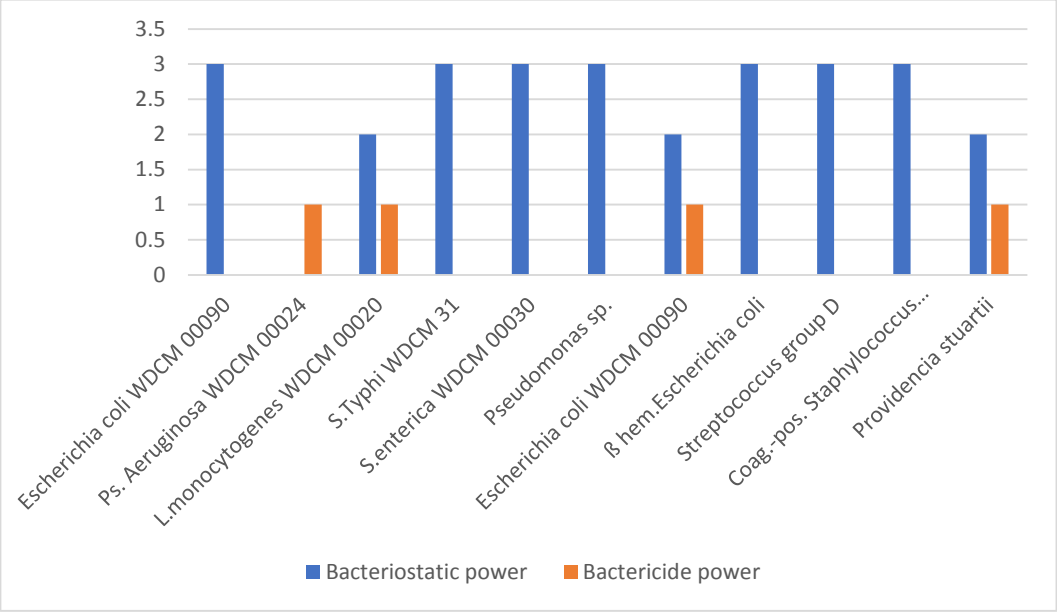


Figure 1. Presentation of White lily extract action on tested microorganisms

Antibacterial activity of White lily extract and its components may vary from partial to total inhibition of bacterial growth, so that bacteriostatic action of White lily extract confirm that fact. Only in four cases White lily extract had bactericide effect.

CONCLUSION

Results of this study confirmed certain antibacterial potency of White lily extract on all tested bacterial strains, but in different range.

-White lily extract showed best antibacterial activity towards clinical izolate ß-hemolytic *Escherichia coli* with inhibition zone from 14.66 mm to 18.33 mm.

-White lily extract showed weaker antibacterial activity towards clinical izolates *Providencia stuartii,* with inhibition zone from 7.66 mm to 8.00 mm in all combinations and coagulase positive staphylococcus with inhibition zone from 7.33 mm to 9.00 mm.

-White lily extract showed weakest antibacterial activity towards *P. aeruginosa* in combination extract: ethanol 1:5 (5.33mm), then towards *E.coli* (6.33 mm) and *Pseudomonas* spp (6.66 mm) in combination extract:ethanol 1:2.

Nowadays, there is a certain tendency for returning to nature and this study presents only introduction in further clinical and laboratory examinations and stimulus for plant preparations usage in treatments of different diseases in humans and animals.

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