

ORIGINAL ARTICLE

Evaluation of Antimicrobial Effect of *Oenothera biennis* by Using Different Concentrations of Extract against *Staphylococcus Aureus* - An experimental study

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ABSTRACT

Background: *Staphylococcus aureus* is a superbug which is infamous for causing wound infections. Unfortunately it is developing resistance quickly against commonly used antibiotics. Antimicrobial drugs effective against methicillin resistant *Staphylococcus aureus* are too expensive to afford by impoverished population in our country. However, herbal medicines are seen to be equally effective with less severe adverse effects. There is need to explore alternative medicines of botanical origin to cure dangerous bacteria. *Oenothera biennis* contains phytochemicals which have antimicrobial activity against some gram negative and gram positive microorganisms.

Objectives: To evaluate the minimum inhibitory concentration of *Oenothera biennis* seed extract against *Staphylococcus aureus* by using different concentrations.

Study Design: In Vitro antibacterial study.

Settings: The study was conducted in microbiology laboratory of Shaikh Zayed Medical Complex, Lahore.

Duration: One year.

Methodology: *Oenothera biennis* extract was prepared in 95% Ethanol in biochemistry laboratory of Pakistan Council of Scientific and Industrial Research (PCSIR) Lahore. *Staphylococcus aureus* isolates were procured from microbiology laboratory and American Type Culture Collection (ATCC) strains used as quality control for MSSA & MRSA, were purchased from Musaji Adam and Sons Karachi (imports@science-ware.com). The antibacterial activity of *Oenothera biennis* seed extract against *Staphylococcus aureus* was tested by deep well broth microdilution and disc diffusion method. Different concentrations of stock solutions (100µg/10µl to 500µg/10µl of DMSO) were used to test antimicrobial effect to establish dose response relationship.

Results: Results were measured and compared according to Clinical and Laboratory Standard Institute. *Oenothera biennis* seed extract inhibited growth of methicillin sensitive and methicillin resistant *Staphylococcus aureus* isolate from laboratory as well as ATCC strains with minimum inhibitory concentration 530µg/10µl solvent. Whereas other concentrations 50 µg/10µl, 100 µg/10µl, 150 µg/10µl, 200 µg/10µl, 250 µg/10µl, 300 µg/10µl, 350 µg/10µl, 400 µg/10µl, 450 µg/10µl and 500 µg/10µl had no effect at all.

Conclusion: *Oenothera biennis* extract inhibits growth of *Staphylococcus aureus* at concentration of 530µg remarkably. This can inhibit both MSSA & MRSA already proved in our study. This could be beneficial as an alternate medicine. However, further research is needed to be conducted for animal study.

Key words: *Oenothera biennis*, ethanolic extract, Methicillin sensitive, Methicillin Resistant *Staphylococcus aureus*, MSSA, MRSA.

INTRODUCTION

Staphylococcus aureus resides at our skin as a normal flora making it first to invade and cause wound infections. Studies show that methicillin resistant *Staphylococcus aureus* (MRSA) is responsible for 70% of hospital acquired wound infections¹. In Pakistan, *Staphylococcus aureus* acquire resistance to penicillin 42% in 2002 and 60.7% in 2016 in elder age group and woefully the trend increase with passage of time^{2,3}.

Staphylococcus aureus is identified by the production of specific enzyme tests⁴. In addition, pigments such as staphyloxanthin, toxins and the outer capsule increase its virulence by weakening immune responses⁵. In case of wound infections, it spreads in the form of a biofilm which is hetero-resistant layer of colonies adding to its virulence⁶. It

is proven that penicillinase and altered penicillin binding proteins coded by *mecA* gene are responsible for resistance. Furthermore, it is classified as methicillin susceptible and resistant isolates known as MSSA and MRSA respectively⁷.

At present, the antibiotics effective against *Staphylococcus aureus* are penicillin such as oxacillin, nafcillin, and cephalosporin such as cefoxitin, cefazolin, ceftaroline and other beta lactam antibiotics such as vancomycin. Vancomycin is an expensive antibiotic and has many harmful effects⁸. Newer economical and antibiotics should be explored against MRSA with less adverse effects.

Herbal medicines are usually harmless due to natural antioxidant properties. Recently, many herbs have been proved to treat *Staphylococcus aureus* infections in the past as well as in modern era⁹.

Oenothera genus grows ample in tropics and well known as evening primrose. Other familiar names are local language it is called "Gul e Fanjani"¹⁰. Oenothera biennis is rich in most of beneficial phytochemicals including catechins, oenothins¹¹, tannins and triterpenes¹². In ancient medicine it was used to cure infections and inflammations. Oenothera biennis contains more concentrations of phenolic compounds as compared to nigella sativa¹³. Water supplementation with oenothera biennis extract effectively decreased incidence of dental caries in animals¹⁴. Consequently, this research was organized to test the antimicrobial activity of oenothera biennis against staphylococcus aureus in vitro.

METHODOLOGY

Herbal preparation of oenothera biennis was used to assess anti-bacterial effective against staphylococcus aureus. Cefoxitin was selected as positive control. Bacterial isolates were obtained from microbiology laboratory, identified as staphylococcus aureus by biochemical tests and were freeze stored. Quality control for methicillin sensitive and resistant ATCC strains were purchased from musaji adam and sons Karachi. oenothera biennis seeds were purchased from Pfizer co. limited that had imported seeds from China. Oenothera biennis extract was prepared at biochemistry laboratory by using 95% ethanol in soxhlet apparatus¹⁶. Extract was stored in air tight sterilized container at room temperature.

American type cell culture ATCC 25923 was used as quality control for methicillin susceptible staphylococcus aureus (MSSA-mecA negative) and ATCC 33591 was used as quality control for methicillin resistant Staphylococcus aureus (MRSA mecA positive)¹⁵. For preparation of stock solution, 0.1% dimethyl sulfoxide (DMSO) was used as solvent as well as for negative control¹⁶. Whatman filter paper was utilized to make antibiotic assay discs with diameter 6 millimeter. The filter paper discs were impregnated with different concentration of extract by using different dilutions of extract stock solution. Inocula were prepared by growing fresh colonies in test tubes containing 3ml of normal saline.

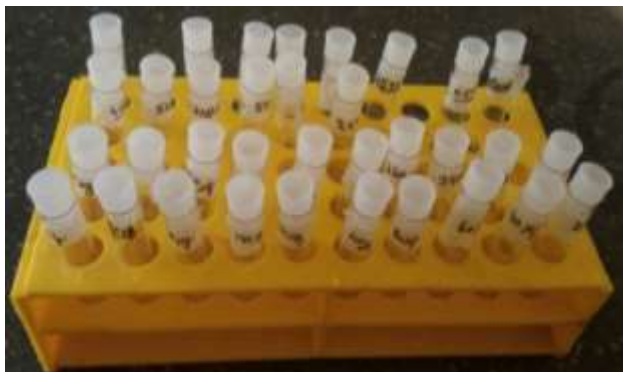


Fig-1: Freshly prepared inocula and different concentrations of oenothera biennis extract ranging from 100µg to 550µg.

Determination of MIC by broth microdilution method: From stock solution, serial dilutions were prepared by dissolving it into dimethyl sulfoxide to get solutions containing extract of 100µg/10µl, 150µg/10µl, 200µg/10µl,

250µg/10µl, 300µg/10µl, 350µg/10µl, 450µg/10µl and 530µg/10µl. All these dilutions were used to determine MIC of the oenothera biennis as shown in figure (Fig-1). Minimum inhibitory concentration of oenothera biennis was evaluated by broth microdilution¹⁷ method. In each tube, 20µl of each dilution was added. The same quantity of inoculum was added to all tubes and incubated at 37°C for 24 hours. Two test tubes containing only the extract were taken as negative control. Each tube was examined for growth and for confirmation inoculated on petri plates and incubated for 24 hours as same is done with control dilutions. The absence of visible bacterial growth on petri plates was taken as minimum inhibitory concentration.

Determination of MIC by disc diffusion tests: In this procedure Kirby Bauer disc diffusion method was used according to CLSI standards¹⁸. McFarland standard was followed regarding turbidity of bacterial suspensions. Whatman filter paper discs containing different concentrations of extract were prepared. Results were recorded by measuring zones of inhibition after 18 hours. The lowest concentration that showed inhibitory zone was noted. Inhibitory zone was observed around the discs containing 350 µg to 530 µg of extract concentration. No zone was seen below 350µg/10µl concentration in disc diffusion method. Minimum inhibitory concentration was observed at 530µg as shown in figure (Fig-2).



Fig-1: Determination of MIC by disc diffusion method: Oenothera biennis seed extract concentration 200µg/10µl, 280µg/10µl, 380µg/10µl, 480µg/10µl and 530µg/10µl.

RESULTS

Oenothera biennis extract was effective against staphylococcus aureus isolates with zone diameter 8-12 millimeter. Zones of inhibition of 8-12mm diameter were noticed at 380µg to 530 µg and minimum inhibitory concentration was observed at 530µg by Kirby bauer disc diffusion method and broth microdilution method.

DISCUSSION

Staphylococcus aureus is mainly responsible for abscess formation¹⁹. Methicillin Resistant Staphylococcus aureus (MRSA) infection is a worldwide concern increasing cost of health care services. Previous studies represent that prevalence of methicillin resistant staphylococcus increased to 23.5% in general public and 10.5% in health sector population. Already effective antibiotics are gradually losing

efficacy due to resistance. Meanwhile, health care personnel prescribing combination therapy which is effective against severe infections. Furthermore, antibiotics always carry the risk of idiosyncratic adverse reactions. It is evident that antibiotic resistance is reduced in areas where antibiotic use is declined. This necessitates the need for economical alternative drugs²⁰.

Large volume of literatures are available for the traditional use of extracts of herbal plants like aerial parts of *hypericum perforatum*, *verbascum mucronatum*, *centaurea iberica*, *salvia fruticosa* against wound infections caused by *staphylococcus aureus* but the concentrations are not specified in a scientific way. However, in our study, we used different concentrations to establish the dose response relationship and concentration dependent killing effect respectively. In our study, we also evaluated minimum concentration which is effective to inhibit bacterial growth.

In present study, we used different concentrations of stock solutions to evaluate antibacterial activity. Based on our results, *staphylococcus aureus* isolates were susceptible to *oenothera biennis* extract with zone diameter starting from 8mm and gradually increased to 12mm by increasing concentration, exhibiting significant zones of inhibition. Bacterial growth inhibition commenced at the concentration of 380µg/10µl solvent and inhibitory zones increased as we increased the dose until 530µg/10µl. Consequently, MIC of *oenothera biennis* extract was observed at concentration of 530µg.

In 2010, Busmann RW et al proved that herbal extracts of *diplostegium sagasteguii* and *lresine herbstii* were effective against *staphylococcus aureus* at concentration of 8mg and 256mg respectively. However, higher concentrations indicated limited antimicrobial activity of these herbs. Another study showed *ageratum conyzoides* and *camellia sinensis* inhibited growth of *staphylococcus aureus* at concentration of 0.42mg and 0.85mg respectively, whereas, we used *oenothera biennis* seed's extract which resulted in a huge difference in MIC concluding that *oenothera biennis* seeds contained the most effective antimicrobial phytochemicals. Furthermore, phytochemicals of *nigella sativa*, *borago officinalis* and *oenothera biennis* seedcake extracts were compared and found out that *oenothera biennis* seeds contained high concentration of polyphenols and flavonoid contents which exhibited maximum antimicrobial properties in plant extracts²¹.

CONCLUSION

Oenothera biennis has remarkable antimicrobial activity in very low concentration as proved in our study.

Limitations: This study was conducted in one hospital with limited number of *staphylococcus aureus* samples. We recommend extending this study to multicentered study to formulate guidelines regarding usefulness of *Oenothera biennis* extract against *staphylococcus aureus* infections.

Recommendations

Further experimental studies are yet to be done to test the antimicrobial effect of *oenothera biennis* by either topical application or oral administration to different groups of animals having infected wounds. Still large scope is

available for studies to see the antibacterial effect of *oenothera biennis* in human beings.

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