



Comparative Antibacterial Study of *Oenothera biennis* Seed Extract with Methicillin and Cefoxitin Against *Staphylococcus aureus* in Vitro

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ABSTRACT

Introduction: The growing incidence of *Staphylococcus aureus* resistance coupled to lagging antimicrobial development has led to fewer effective antibiotics against it. Newer anti staphylococcal phytochemical compounds are imperative with detailed validation against different antibiotics. Emerging in vitro data has noted a resistance modifying effect of certain phytochemicals in combination with antibiotics. Our published research on *Oenothera biennis* has revealed its anti-staphylococcal potential in comparison to the gold standard vancomycin and therefore the need to further explore efficacy as opposed to other commonly used antibiotics. **Aims & Objectives:** To compare the antibacterial activity of *Oenothera biennis* seed extract with methicillin and cefoxitin and to test their synergism against *Staphylococcus aureus*. **Place and duration of study:** The study was conducted in Microbiology Laboratory of Shaikh Zayed Medical Complex, Lahore from April 2015 to January 2016. **Material & Methods:** *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 collected from clinical specimens of microbiology laboratory Shaikh Zayed Hospital. American Type culture Collection (ATCC 25923) and (ATCC 29213) were used as quality control for MSSA&MRSA respectively. The antibacterial activity of *Oenothera biennis* seed extract against *Staphylococcus aureus* was compared to that of cefoxitin & methicillin by Kirby Bauer disc diffusion method. Results were measured and compared according to Clinical and Laboratory Standard Institute (CLSI). **Results:** *Oenothera biennis* seed extract has significant antibacterial activity in comparison with Methicillin and Cefoxitin against *Staphylococcus aureus* with P.value<0.001. **Conclusion:** Our study showed that *Oenothera biennis* seed extract has superior antibacterial activity than methicillin and cefoxitin against *Staphylococcus aureus*. Unfortunately no synergism was observed amongst methicillin, cefoxitin and *Oenothera biennis* seed extract.

Key words: *Oenothera biennis*, Seed extract, Methicillin Resistant *Staphylococcus aureus*, MSSA, MRSA, antibacterial activity.

INTRODUCTION

Despite improvements in scientific research antimicrobial resistance is rising. Recent researches have shown that efflux of drug by multi-drug resistance (MDR) pumps is an important cause of resistance in *Staphylococcus aureus*¹. In vitro intrinsic resistance to penicillin was detected in

Staphylococcus aureus in 1965 by Jevons before it was reported clinically². The 2014 WHO report on antibiotic resistance states that fastidious management can lead to a disaster in future by development of microorganisms which would be resistant to all current antimicrobial drugs. Health professionals need awareness of fatal consequences arising from even mild infections in future³. However, we may save humanity from this

impending apocalypse by mandating prescriptions, carefully optimizing existing antibiotic therapy as well as continually developing and exploring newer drugs from different sources⁴. The need for developing economical alternatives with mild adverse effects also remains⁵. Some recent studies have noted interestingly that using plant compounds in combination with conventional drugs could modify resistance patterns, enhance efficacy of drugs and prevent them from becoming obsolete⁶. Methicillin, the first penicillin to be effective against *Staphylococcus aureus* and cefoxitin a second generation, semi-synthetic cephalosporin are now only partially effective against many gram positive organisms including *Staphylococcus aureus*⁷. Ceftaroline a newer more expensive cephalosporin was initially effective against *MRSA*, but resistance has emerged here as well⁸.

An ancient Chinese herbal medicine *Oenothera biennis* (Evening Primrose) oil is a constituent of “cellasene” a product marketed all over the world to treat cellulitis⁹.

Different phytochemical studies have shown *Oenothera biennis* seed extract to contain bioactive phenolic fractions¹⁰. Our previous study supported these findings and revealed significant antibacterial activity of *Oenothera biennis* seed extract compared to vancomycin¹⁹. The present study was designed to further evaluate its comparative and synergistic antibacterial activity to methicillin and cefoxitin against *Staphylococcus aureus* in vitro.

MATERIAL AND METHODS

Chemicals & Instruments: Mueller Hinton agar (OXOID), CLED agar with indicator(OXOID), 0.2% triphenyltetrazolium chloride (TTC) dye, dimethyl sulfoxide (DMSO), Whatman filter paper no. 41 (Sigma Aldrich), incubator 35°C(Sigma Aldrich), petri plates (90mm), 0.2µm filter.

Inclusion Criteria: Both *MSSA* & *MRSA* *Staphylococcus aureus* were included.

Exclusion Criteria: Coagulase negative *Staphylococci* were excluded.

Design: In vitro antibacterial study.

Sample Size: 70 *Staphylococcus aureus* isolates were used for study.

Positive Control Drugs: Standard antibiotic discs of Methicillin (ME-10µg), Cefoxitin (FOX-30µg) (Getz) were used for comparison and also as positive control.

Preparation of Media: Mueller Hinton and nutrient agar was prepared in microbiology laboratory according to instructions leaflet of (OXOID).

Negative Control: 0.1% dimethyl sulfoxide (DMSO) was used as negative control with no zone of inhibition.

Quality Control: American Type Culture Collection ATCC 25923 *MSSA*, ATCC 29213 *MRSA* were used for quality control.

Antimicrobial activity: Antimicrobial activity of *Oenothera biennis* seed extract was determined by Kirby-Bauer disk diffusion method according to CLSI standards¹¹.

Testing Synergism: For testing synergism double disc synergy was employed similar to the method used to detect extended spectrum beta lactamase (ESBL). Here the distance between the drug discs and *Oenothera biennis* seed extract discs in the petri plates was maintained at less than 20 mm. After placing the discs, plates were closed with lids and were kept upside down (inverted position) in the incubator at 35°C for 18-24 hours. Synergism plates were observed for any increase in zone diameter or the enlarged combine zone between drug and *Oenothera biennis* seed extract discs.

Statistical analysis:

One way ANOVA and Post hoc Tukey’s test were used.

RESULTS

In this study bacterial samples were taken from patients from different wards. The mean age of the patients was 38.3 ± 18.5 years¹⁹. A relevant drug history was taken from patients and was found that most of the patients had been using Penicillin and Cephalosporin. Most of patients had taken Amoxicillin, Cefotaxime and Ceftriaxone. Minimum Inhibitory Concentration of *Oenothera biennis* seed extract was found to be 320-340ug by micro broth dilution & Kirby-Bauer disk diffusion method according to CLSI standards¹⁹.

All the isolates were susceptible to *Oenothera biennis* seed extract with average zone diameter of 19 mm. No significant synergism was observed. All 70 isolates of *Staphylococcus aureus* were resistant to methicillin and exhibited no zone of inhibition. Regarding Cefoxitin 40 cases were resistant and 30 cases were susceptible with mean zone of inhibition 15.57 ± 8.08 mm.

In further detailing amongst those resistant isolates, 22% showed absolute resistance similar to methicillin with no zone of inhibition, whereas 28% isolates exhibited mean zone value 10-16mm, 7% of the resistant isolates exhibited re-growth. (Table-1).

Drug	Resistant n(%)	Sensitive n (%)	No Response n (%)	Re-growth n (%)	Total
Methicillin	70 (100%)	0 (0%)	0 (0%)	0 (0%)	70 (100%)
Cefoxitin	20 (28%)	30 (42%)	15 (22%)	5 (7%)	70 (100%)
<i>Oenothera biennis</i> Seed Extract	0 (0%)	70 (100%)	0 (0%)	0 (0%)	70 (100%)
p-value	< 0.001				
Chi-Square test value = 208.13					

Table-1: Frequency of Resistance and Sensitivity of Drugs against *Staphylococcus aureus*

Oenothera biennis seed extract had significantly high antibacterial activity than Cefoxitin with mean difference of 0.530 and -3.17 respectively. For multiple comparisons, post hoc Tukey test was applied and *Oenothera biennis* seed extract was found to have anti-staphylococcal activity statistically significantly high as compared to Methicillin and Cefoxitin with mean difference of -18.74, -3.17, -3.70¹⁹ respectively (Table-2). One way analysis of variance (ANOVA) test was also applied for comparison (Table-3).

Multiple Comparison				
Groups		Mean Difference	Std. Error	P-value
Methicillin	Cefoxitin 30µg	-15.57*	0.696	<0.001
Methicillin	<i>Oenothera biennis</i> seed Extract 340µg	-18.74*	0.696	<0.001
Cefoxitin	<i>Oenothera biennis</i> seed Extract 340µg	-3.17*	0.696	<0.001
Vancomycin	<i>Oenothera biennis</i> seed Extract 340µg	-3.70*	0.696	<0.001

*The mean difference is significant at the 0.05 level.

Table-2: Pair wise comparison of mean inhibition zone among drugs.

Drug	N	Mean ± SD	Minimum	Maximum	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Methicillin 10µg	70	0.00 ± 0.00	0.00	0.00	0.00	0.00
Cefoxitin 30µg	70	15.57 ± 8.08	0.00	25.00	13.65	17.50
<i>Oenothera biennis</i> seed extract 320-340µg	70	18.75 ± 0.96	14.70	21.00	18.52	18.98
Total	280	12.34 ± 8.35	0.00	25.00	11.36	13.32

Table-3: One way ANOVA test for comparison of mean inhibition zones.

DISCUSSION

Staphylococcus aureus is notorious for causing a variety of illnesses, from minor skin infections to life-threatening diseases and approximately 30% of the human population is colonized with it. Unfortunately its resistance is rapidly increasing throughout the world as well as in Pakistan and gradually the conventional antibiotics are losing efficacy¹³.

We are unable to stem the development of antibiotic resistance as a study done by Stephen S Gillespie concluded that mutation is an ongoing process, causing approximately 0.0033 mutations in each bacterial genome replication²⁰.

In our study among 70 isolates of *Staphylococcus aureus*, 58.6% were male and 41.1% were female. The mean age was 38.3 ± 18.5 years and more elderly patients were infected with *Staphylococcus aureus*. The reason can be that the elderly persons have low immunity, chronic ailments and more exposure to antibiotics¹⁴. However, the prevalence of *Staphylococcus aureus* was found to be 53% in surgical wards reflecting a need to focus on the proper sterilization of instruments and encouraging use of effective disinfectants at surgical floors¹⁵.

Our study showed 100% resistance to Methicillin. These results are similar to a study by Hussain M, Basit A, Khan A, et al in Kohat, Pakistan¹⁶ which showed 100% penicillin resistance in *Staphylococcus aureus* isolates. This can be attributed to a rise in MRSA prevalence.

Regarding Cefoxitin, 40 cases (57%) were resistant and 30 cases (43%) were susceptible with mean zone of inhibition 15.57 ± 8.08 mm. Previous researches have proved that cefoxitin can detect *mecA* gene in laboratory and studies have shown that results compared with the famous laboratory test Polymerase Chain Reaction (PCR) showed significant specificity¹⁷. According to a study in Pakistan cephalosporins are the most common antibiotics to be misused causing an increase in the prevalence of resistance¹⁸.

All the isolates were susceptible to *Oenothera biennis* seed extract with mean zone of inhibition 18.75 ± 0.96 and no re-growth was observed¹⁹.

One way ANOVA test was applied for comparison and a significant difference in mean inhibition zone was found among drugs and *Oenothera biennis* seed extract with p -value < 0.001 . Therefore substantial anti-staphylococcal effect is exhibited by *Oenothera biennis* seed extract comparable to methicillin and cefoxitin.

Synergism was tested between *Oenothera biennis* seed extract and standard drugs (vancomycin¹⁹, cefoxitin and methicillin) against *Staphylococcus aureus*. No synergism was observed between the drugs and plant extract. The reason may have been non sequential antibacterial actions in *Oenothera biennis* seed extract, cefoxitin and methicillin which resulted in an inability to boost each other's effect.

CONCLUSION

This study showed significant anti-staphylococcal activity of *Oenothera biennis* seed extract with MIC value $320 \mu\text{g}$ ¹⁹. At this concentration it was found to be slightly more efficacious than Cefoxitin and methicillin against *Staphylococcus aureus* with potential against other gram positive and gram negative bacteria for its antibacterial activity.

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