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Comparative Antibacterial Study of *Oenothera biennis* Seeds Extract and Vancomycin Against *Staphylococcus aureus* in Vitro

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ABSTRACT

Introduction: Resistant topical Staphylococcus aureus wound infections are becoming increasingly common and life threatening worldwide. Vancomycin remains the drug of choice but lately international reports of resistance towards it have emerged with a looming threat in Pakistan as well. New phytopharmaceuticals need to be investigated to counter this phenomenon. Oenothera biennis seeds extract could be a potential source of the next generation antistaphylococcal drugs. **Aims and Objectives:** To determine the Minimum Inhibitory Concentration (MIC) of *Oenothera biennis seeds* extract in comparison to vancomycin against *Staphylococcus aureus*. **Design:** *In vitro* antibacterial study. **Place and Duration of Study:** The study was conducted in Microbiology Laboratory of Shaikh Zayed Medical Complex, Lahore from March 2015 to February 2016. **Material and Methods:** *Oenothera biennis seeds* extract was prepared in biochemistry laboratory. The Minimum Inhibitory Concentration (MIC) of *Oenothera biennis seeds* extract against Methicillin Resistant *Staphylococcus aureus* was compared to that of vancomycin by Kirby Bauer disc diffusion method. Results were measured and compared according to Clinical and Laboratory Standard Institute (CLSI) guidelines of microbiology used worldwide. **Results:** *Oenothera biennis seeds* extract had antibacterial activity against all isolates of *Staphylococcus aureus (MRSA)* with MIC value of 320-340µg as compared to the MIC value of <3µg for vancomycin, with significant P- value <0.001. **Conclusion:** Our study showed that *Oenothera biennis seeds* extract had less potent, but significant antibacterial activity comparable to Vancomycin against Methicillin Resistant *Staphylococcus aureus*.

Key words: *Oenothera biennis*, *Staphylococcus aureus*, MRSA, ethanolic, antibacterial activity, *in vitro*.

INTRODUCTION

Skin is the natural protection barrier of human body and its damage can lead to bacterial invasion and infection[1]. Topical wound infection is very common in patients with burns and chronic skin disorders[2]. Wound infection can be caused by multiple organisms including gram positive and gram negative bacteria with a predominance of gram positive bacteria. Frequent wound infecting bacteria are *Pseudomonas aeruginosa, Enterococcus* species, coliform bacteria, *Staphylococcus epidermidis* and *Staphylococcus aureus*[3].

Staphylococcus aureus is responsible for dermatological diseases such as impetigo, cellulitis and carbuncle[4]. Secondary infections in skin diseases are commonly caused by Methicillin Resistant *Staphylococcus aureus* (MRSA) such as seen in scabies and dermatitis[1]. *Methicillin Resistant Staphylococcus aureus (MRSA)*, is a superbug responsible for delayed recovery in both acute and chronic wounds[5]. It is emerging as multidrug resistant pathogen and it may come out as a great burden on the nations in future if not taken seriously[6]. According to an annual report of US Centre for Disease Control, prevalence of MRSA was 60% and caused 80,000 deaths in USA[7].

Current treatment modalities involve the usage of common anti-staphylococcal drugs such as penicillins, cephalosporins and vancomycin. Out of all these drugs, vancomycin is the drug of choice against Methicillin Resistant *Staphylococcus aureus* (MRSA) but it is too expensive to be afforded by the low income wage earners[8].

Phytotherapy holds promise in this regard as herbal medicines provides an alternative, economical and potential cure with mild adverse effects[9]. Compounds of plant origin could also modify resistance patterns thus enhancing efficacy of drugs that were formerly resistant. This can prevent common drugs from becoming obsolete[10]. Different studies have shown that some plants have antibacterial activity against *Staphylococcus aureus* such as *Onosmabracteatum* (gaozuban), *Menthapiperita* (peppermint) and possibly *Oenothera biennis* (Evening primrose)[11]. In traditional Chinese medicine system genus *Oenothera* was used in treatment of many diseases including skin inflammations[12]. Evening primrose oil was a part of the product "cellasene" marketed all over the world to treat cellulitis which is mainly caused by *Staphylococcus aureus*[13].

Oenothera biennis was studied to have phenolic fractions which have effective antibacterial activity against *Streptococcus mutans* causing dental caries in rats[14]. Similar potential against *Staphylococcus aureus* could exist in *Oenothera biennis seed*s. Therefore, this study was designed to evaluate the antibacterial activity of *Oenothera biennis seeds* extract and its comparison with vancomycin against *Staphylococcus aureus in vitro*.

MATERIAL AND METHODS

Chemicals and Instruments: Mueller Hinton agar and CLED agar with indicator were purchased from Oxoid Pak. Dimethyl sulfoxide (DMSO), Whatman filter paper no.2, incubator 35˚C were obtained by Sigma Aldrich.

Drugs and positive control: Standard antibiotic disc of Vancomycin (Getz Pharma) were used for comparison and also as positive control.

Negative control: Whatman filter paper discs were impregnated with 0.1% dimethyl sulfoxide (DMSO) as negative control.

Sample size: 70 *Staphylococcus aureus* isolates.

Collection of plant specimen: *Oenothera biennis seed*s were purchased from local market.

Preparation of ethanolic extract of *Oenothera biennis seeds*: *Oenothera biennis seeds* extract was prepared in biochemistry laboratory at the Pakistan Council of Scientific and Industrial Research (PCSIR) Lahore.

Inclusion criteria: Only *Staphylococcus aureus* isolates were confirmed and included in research.

Exclusion criteria: DNase negative and Coagulase negative Staphylococci were excluded.

Methodology for testing Anti-bacterial activity: Antimicrobial activity of *Oenothera biennis seeds* extract was determined by Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) standards[15].

Interpretation of results: The diameters of zones of inhibition were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) recommendations (Fig-2). Relevant basic clinical data was collected on a proforma for any significant correlation.

Minimum Inhibitory Concentration (MIC) of *Oenothera biennis seeds* extract: MIC was determined by broth Macro dilution method and disc diffusion method. Stock solution was prepared and serial dilutions of *Oenothera biennis seeds* extract were prepared in test tubes with concentrations ranging from 20µg/10µL-500µg/10µl of dimethyl sulfoxide (DMSO) as a solvent. Serial dilution of stock solution was prepared by dissolving into DMSO to get solutions containing extract of 20µg/10µl of DMSO, 40µg/10µl, 60µg/10µl, 80µg/10µl 100µg/10µl, 120µg/10µl, 140µg/10µl, 160µg/10µl, 180 to 200µg/10µl, 300µg/10µl, 400µg/10µl, 500µg/10µl. All these dilutions were used to determine MIC of the *Oenothera biennis seeds* Extract (Fig-1).

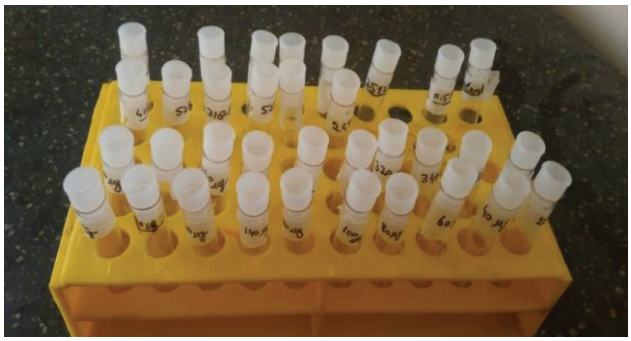


Fig-1: Test tubes containing trypticase soya broth (2ml), freshly prepared inocula (10µl), different concentrations of *OBSE* ranging from20µg/10µl - 500µg/10µl

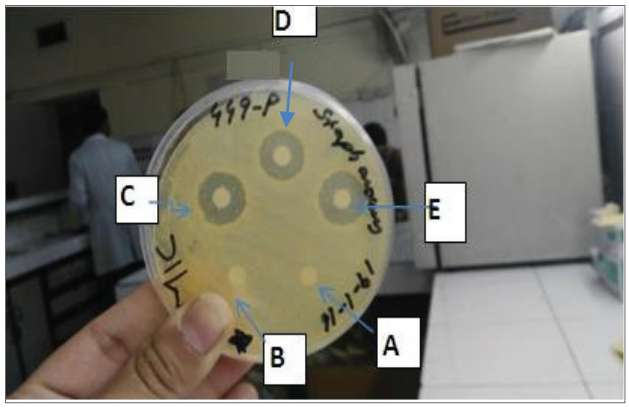


Fig-2: Determination of MIC by disc diffusion method: *Oenothera biennis seeds* extract concentration 280µg/10µl DMSO (0.1%) (A), 300µg/10µl (B), 320µg/10µl (C), 340µg/10µl (D), 360µg/10µl (E).

Preparation of DMSO disc as negative control: Discs were impregnated with 0.1% DMSO solution as negative control had no zone of inhibition.(Fig-3)

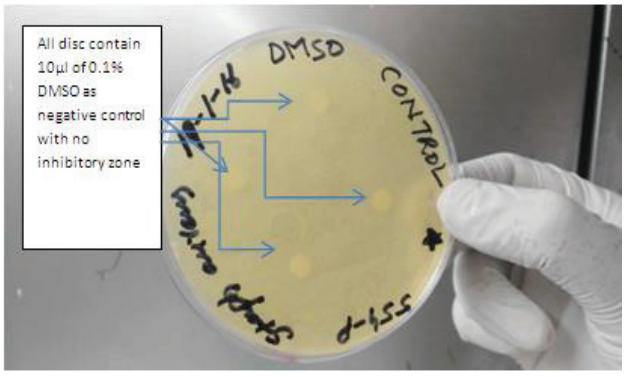


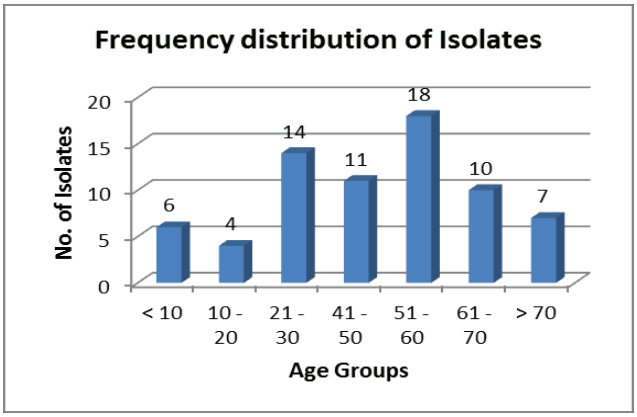
Fig-3: Filter paper discs of dimethyl sulfoxide (DMSO) 0.1%, showing no zone of inhibition.

Statistical analysis:

Data was analyzed using SPSS version 20. One way ANOVA test was applied for comparison of mean zones of inhibition of extract and vancomycin. A p-value of <0.05 was considered statistically significant.

RESULTS

Among the 70 *Staphylococcus aureus* isolates, 41 samples were from males and 29 samples were from females and the mean age was found to be 38.3 ± 18.5 years (Graph-1).



Graph-1: Frequency distribution of *Staphylococcus aureus* in different age groups.

MIC of *Oenothera biennis seeds* extract was found to be 320-340µg. All the isolates were susceptible to vancomycin and *Oenothera biennis seeds* extract. A sample picture of zone diameter of seed extract and vancomycin disc is shown in Fig-4.

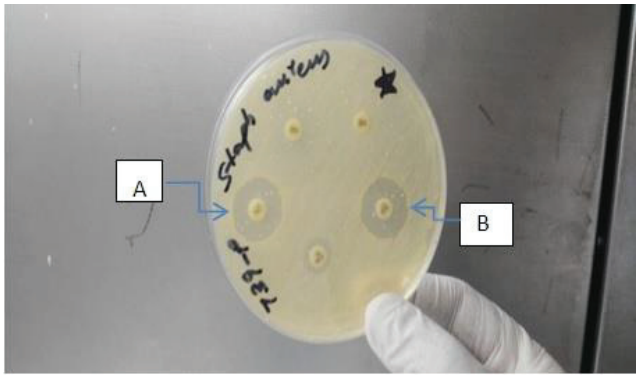


Fig-4: *Oenothera biennis seeds* extract disc (A) and vancomycin disc (B) showing clear zones of inhibition.

Drug	Resistance n (%)	Sensitive n (%)	No Response n (%)	Re-growth n (%)	Total
Vancomycin 30µg(St. Disk)	0 (0%)	70 (100%)	0 (0%)	0 (0%)	70 (100%)
***Oenothera Biennis* Seed Extract 340µg**	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*

All isolates were susceptible to vancomycin (VA-30µg) with zone diameter ranging from 7-15mm by disc diffusion method and MIC value<3µg by macro broth dilution method (Table-1). All isolates were susceptible to *Oenothera biennis seeds* extract with zone diameter ranging from 18-20mm with MIC value 340µg/10µl of solvent 0.1% dimethyl sulfoxide (DMSO) (Table-2).

Drug	N	Mean ± SD(mm)	Minimum	Maximum	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Vancomycin	70	15.04 ± 1.33	9.30	17.0	14.7	15.3
***Oenothera biennis* seed extract**	70	18.75 ± 0.96	14.70	21.0	18.5	18.9
Total	140	12.34 ± 8.35	0.00	25.0	11.3	13.3

Table-2: Comparison of mean zones of inhibition of vancomycin and *Oenothera biennis seeds* extract against *Staphylococcus aureus.*

DISCUSSION

Humans have been suffering from microbial infections since the beginning of humanity and wound infections have been a leading cause of debility in the community as well as hospitals in Pakistan[16]. *Staphylococcus aureus* is a bacterium notorious for causing many skin diseases and one of alarming feature is that it develops resistance rapidly[17]. Methicillin Resistant *Staphylococcus aureus* (MRSA) infection is an economic burden globally[18]. In Pakistan resistance of *Staphylococcus aureus* is multiplying and the prevalence[19,20] of MRSA increased from 5% in 1989 to 43 % in 2002. It could be due to excessive use of antibiotics by general practitioners for mild infections during this period and unfortunately this trend still continues.

In our study 58.6% samples were from males and 41.1% were from females with a mean age of 38.3 ± 18.5 years and more elderly patients were infected with *Staphylococcus aureus* as shown by a similar study in 2005 in Norway[21]. Drug history from our patients noted that most of them despite using penicillins and cephalosporins for their wound condition were finally compelled to be hospitalized. In our study vancomycin susceptibility was 100% with mean zone of inhibition 15.04±1.33mm and MIC value <3µg by macro broth dilution method. This confirmed the importance of vancomycin as the standard antistaphylococcal drug and showed that fortunately in our area vancomycin resistance hadn't developed yet. This was comparable to a multicenter study conducted in Pakistan in 2002 and 2013 in which no vancomycin resistance was detected in *Staphylococcus aureus* isolates[20,22]. However, there is need to carefully monitor vancomycin use and study prevalence of sensitivity patterns of *Staphylococcus aureus* every year to identify resistant genes early.

Based on our results in comparison surprisingly all isolates of *Staphylococcus aureus* were susceptible to *Oenothera biennis seeds* extract with zone diameter ranging from 18-20mm with MIC value 340µg/10µl .Most importantly not a single isolate showed re-growth with *Oenothera biennis seeds* extract. The anti-staphylococcal effect exhibited by *Oenothera biennis seeds* extract was comparable to that of vancomycin which is an expensive drug.

CONCLUSION

This study showed significant anti-staphylococcal activity of *Oenothera biennis seeds* extract against *Staphylococcus aureus* and it could be used as an economical alternative medicine in future.

Future Recommendations: Further studies should be done to isolate and purify the phenolic fractions of seed extract which would possibly result in a lower MIC and greater efficacy against *Staphylococcus aureus* and other wound infecting organisms. As results of in vitro studies have certain limitations and are not applicable to in vivo effects, there is need to perform an in vivo study as well.

REFERENCES

1. Cardona AF, Wilson SE. Skin and soft-tissue infections: a critical review and the role of telavancin in their treatment. Clin Infect Dis. 2015 Sep 15;61Suppl 2:S69-78. doi:10.1093/cid/civ528.
2. Bacterial and fungal profile of burn wound infections in Tertiary Care Center Mundhada Sapana G, Waghmare Prakash H, Rathod Prachala G, Ingole Kishore V, 2015, Vol: 23, Issue Number: 1;71-75.
3. Bowler PG, Duerden BI, Armstrong DG, Wound microbiology and associated approaches to wound management. Clin.Microbiol. Rev. 2001,14(2):244-269.
4. Lowy. F.D: Medical progress: *Staphylococcus aureus* infections. N Engl J Med. 1998.339:520-32.
5. Alebachew T, Yismaw G, Derabe A, Sisay Z. *Staphylococcus aureus* Burn Wound Infection among patients attending Yekatit 12 Hospital Burn Unit, Addis Ababa, Ethpia. Ethop J Health Sci. 2012; 22(3):209-213.
6. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial blood stream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004; 39:309–17
7. Klevens RM, Edwards JR, Richards CL. et al. Estimating healthcare-associated infections and deaths in U.S. hospitals, *Public Health Rep*. 2007;122(2):160-166.
8. Rybak MJ, Lomaestro BM, Rotschafer JC, et al. Vancomycin therapeutic guidelines: a summary of consensus recommendations from the Infectious Diseases Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists. Clin Infect Dis 2009; 49:325–7.
9. A. Al-Habib, E. Al-Saleh, A. M. Safer and M. Afzal, "Bactericidal effects of grape seed extract on methicillin resistant *Staphylococcus aureus* (MRSA), Journal of Toxicological Sciences, 2010; vol. 35, no. 3, pp. 357-364.
10. Muroi, H. and Kubo, I. Antimicrobial activity of anacardic acids and totarol, alone and in combination with methicillin, against methicillin resistant *Staphylococcus aureus*. J. Appl. Bacteriol 1996, 80: 387-394.
11. Gibbons, S: Anti-staphylococcal plant natural products. The Royal Society of Chemistry. Journal of Natural Product Reports 2004. 21:263-277.
12. Shukla YN, Khanuja SPS. Chemical, biology and agro technological studies on the genus Oenothera- a review. J Med Aromat Plant Sci. 2004; 26:527-533.
13. Lis-Balchin M. Parallel placebo controlled clinical study of a mixture of herbs sold as remedy for cellulite. Phytother Res. 1999; 13(7): 627-629.
14. Matsumoto Nakano M, Nagayama K, Kitagori H, Fujita K, Inagaki S, Takashima Y, Tamesada M, Kawabata S, Ooshima T, Inhibitory effects of *Oenothera biennis* (evening primrose) seed extract on *Streptococcus mutans* induced dental caries in rats. doi:10.1159/000323376., Epub 2011 Feb 11,2011; 45(1): 56-63.
15. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approval Standard fifth edition. Wayne (PA) 2012: CLSI;2012.
16. N Muhammad, Adil M, Naz SM, Abbas SH, Khan MZUI, et al. Resistance and sensitivity pattern of *Staphylococcus aureus*; A study in Lady Reading Hospital Peshawar. J Postgrad Med.Inst.2013;27(1):42-7.
17. Heather K. Allen, Justin Donato, Helena Huimi Wang, Karen A. Cloud-Hansen, Julian Davies & Jo Handelsman. Call of the wild: antibiotic resistance genes in natural environments. N Rev Microbiol 2010,vol.8, 251-259.
18. Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, Lessa FC, Lynfield R, Nadle J, Petit S, Ray SM, Schaffner W, Townes J, Fridkin S. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. JAMA Intern Med. 2013;3(21).
19. Ashiq B, Tareen AK. Methicillin resistant Staphylococcus aureus in a teaching hospital of Karachi-a laboratory study. J Pak Med Assoc 1989; 39:6-9.
20. S. Hafiz, A. N. Hafiz, L. Ali, A. S. Chughtai, B. Memon, A. Ahmed, S. Hussain, G. Sarwar, G. Sarwar, T. Mughai, A. Awan, K. Zaki, A. Fareed. Methicillin resistant *Staphylococcus*

aureus: a multicenter study. 2002; JPMA 52: 31.
21. Hanssen M A, Fossam A, Mikalsen J, Halvorsen S D, Bukholm G, Johana U. Dissemination of community acquired methicillin resistant *Staphylococcus aureus* clones in Northern Norway. J Clin Microbiol 2005;43(5):2118-2124.
22. Hussain M, Basit A, Khan A, Rahim K, Javed A, Junaid A, Munir S, Niazi HR, Sohail M, Hussain T. Antimicrobial Sensitivity Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Hospitals of Kohat District, Pakistan. J. Inf. Mic. Bio 2016.1: 13-16.

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