Allied Medical Research Journal
Vol:3 No.4: 05-13

Original Article

Evaluation of the Antimicrobial Activity of *Chlorella vulgaris* Against MDR Bacteria Isolated from Diabetic Wound Pus Samples

Ahmad Naeem Sajed[1], Mohsin Gulzar Barq[1], Muhammad Waseem[2], Muhammad Ahsan Murtaza[2], Muhammad Tayyab[3], Abdul Nafeh[4]

[1]*University of Central Punjab, Lahore, Pakistan*
[2]*Superior University, Lahore, Pakistan*
[3]*Lahore Garrison University, Lahore, Pakistan*
[4]*Sialkot Medical College, Sialkot, Pakistan*

ABSTRACT

Background: The aim of this study was to isolate and characterize pathogenic microbes from the pus of diabetic patients and to evaluate the antibiotic susceptibility of multidrug-resistant (MDR) isolates along with their resistance patterns against the microalgae *Chlorella vulgaris* extract.

Methods: This hospital-based biphasic research study (cross-sectional and experimental) included 100 pus samples from diabetic patients. Out of these, 10 isolates were identified as multidrug-resistant (MDR) and were subsequently subjected to further characterization. Two methods were employed to evaluate the antimicrobial activity of the microalgae *Chlorella vulgaris*. Since the Kirby-Bauer disc diffusion method did not provide sufficient results, a turbidity assay was performed in an ELISA 96-well microtiter plate to evaluate the inhibitory effect of microalgae *Chlorella vulgaris* extract on bacterial growth. To assess the biofilm-forming ability of pus isolates and to evaluate the inhibitory potential of *Chlorella vulgaris* extract against their biofilms standard microtiter plate assays were performed.

Results: The *Chlorella vulgaris* extract (CVE) exhibited species-dependent antibiofilm activity against pus isolates from diabetic patients. The strongest % inhibition at MIC with algal extract was observed in *Acinetobacter species* (48.28%) and *Streptococcus pyogenes* (42.12%), while the weakest response was noted in *Klebsiella pneumoniae* (27.48%). Overall *Chlorella vulgaris* extract (CVE) demonstrated significant inhibitory potential ($p < 0.05$) with comparatively stronger effects against certain Gram-negative and selected Gram-positive bacteria.

Conclusion: Microalgae *Chlorella vulgaris* extract exhibited antimicrobial and anti-biofilm activity against MDR isolates from diabetic patients with the strongest effects on *Acinetobacter species* and *Streptococcus pyogenes* indicating its potential as a natural therapeutic requiring further mechanistic and in vivo validation.

Keywords: *Antimicrobial, Chlorella vulgaris extract, Diabetes, Microalgae, Pyogenic infections, Topical antimicrobial.*

Received: January 25, 2025**; Revised:** September 22, 2025**; Accepted:** October 21, 2025
Corresponding Email: L1S23PHMR0001@ucp.edu.pk
DOI: https://doi.org/10.59564/amrj/03.04/003

INTRODUCTION

Diabetes is a multifactorial disease linked to organ damage and high blood sugar which impair healing and promote pus-forming (pyogenic) infections[1]. The infections are broadly classified as pyogenic involving pus-producing bacteria or non-pyogenic, marked by systemic inflammation at wound sites[2].

Abscesses develop on the skin or inside the body when exposed to pathogenic bacteria such as *Staphylococcus aureus* which release toxins that damage tissues resulting in the formation of pus[3,4]. Antibiotic selection depends on pharmacodynamics, pharmacokinetics and the infectious process of target bacteria[5]. However excessive and improper use of antibiotics has led to resistance particularly in gram-negative species. Methicillin-resistant *Staphylococcus aureus* (MRSA), a gram-positive bacterium is strongly linked to pyogenic

infections[6]. Resistance factors can also transfer between species and multidrug-resistant strains are frequently found in immunosuppressed patients including those with diabetes[7]. Antibiotic-resistant bacteria complicate treatment raising morbidity, mortality and healthcare costs especially in immunosuppressed patients. Antimicrobial resistance (AMR) hinders infection management including in diabetic patients highlighting the urgent need for novel therapies and effective infection-control strategies[8]. *Chlorella vulgaris* is commonly employed as a pathogen-removing agent in wastewater treatment and has also been reported to exhibit antimicrobial activity against opportunistic bacteria[9,10].

This research study aimed to isolate and characterize pathogenic bacteria from pus samples of diabetic patients to determine their resistance patterns and biofilm-forming abilities and to evaluate the antibacterial activity of *Chlorella vulgaris* extract (CVE) using inhibition zone and MIC assays against both planktonic and biofilm-associated isolates. We hypothesized that the microalgal extract of CVE would exhibit significant antimicrobial and antibiofilm activity against multidrug-resistant (MDR) bacterial isolates from diabetic patients owing to its bioactive compounds including phenols, alkaloids and fatty acids.

METHODOLOGY

Study Design

This hospital-based research study combined cross-sectional isolation and characterization of pathogenic bacteria from diabetic patients with experimental evaluation of the antibacterial potential of CVE against planktonic and biofilm-forming strains.

Inclusion and Exclusion Criteria

This research study included patients with clinically diagnosed diabetes who presented pus samples from wound infections. We excluded non-diabetic patients those who had received antibiotic treatment within the last 7 days and those with insufficient or contaminated samples.

Ethical Considerations

All patients were examined and investigated after obtaining informed written consent. Adequate privacy and the presence of a chaperone were ensured throughout the procedures. Ethical approval for this study was obtained from the Ethical Review Board of Allama Iqbal Medical College/Jinnah Hospital, Lahore.

Collection of Chlorella vulgaris Samples

The samples of microalgae *Chlorella vulgaris* were collected from Rohi Nala wastewater near Bahria Town, Lahore and cultivated in Bold's Basal Medium (BBM) containing essential salts to promote their growth and prepare for antimicrobial activity testing.

Gathering and Handling of Pus Samples from Individuals with Diabetes

The 100 samples were collected from diabetic patients admitted to the surgical, emergency and medical wards of Jinnah Hospital, Lahore. The samples were aseptically collected using sterile syringes and swabs, transported to the microbiology laboratory and processed for further testing.

Preparation of Organic Algal Extract

An aqueous extract of microalgae *Chlorella vulgaris* was prepared to evaluate its antibacterial activity. The harvested algal biomass was suspended in distilled water and bioactive compounds were extracted through sonication followed by continuous stirring at a controlled temperature. The resulting suspension was centrifuged to remove cellular debris and the supernatant was subsequently filtered to eliminate algal cells yielding a clear extract suitable for antibacterial assays.

Culture Method and Identification of MDR Bacterial Strains

Blood agar (BA), nutrient agar (NA) and MacConkey agar (MA) media were used for bacterial culture and sensitivity testing. Blood agar (BA) plates were incubated in an enriched carbon dioxide candle jar while nutrient agar and MacConkey agar plates were incubated at 37°C for 24-48 hours. Single isolated colonies obtained from one medium were further inoculated onto the corresponding media for purification.

Culture and morphological characteristics were recorded on MacConkey agar and hemolysis patterns were observed on blood agar. The isolates were subsequently subjected to antibiotic susceptibility testing and strains resistant to three or more classes of antibiotics were identified as MDR bacteria by the Kirby-Bauer disc diffusion method.

Antibacterial Assay of Microalgae Chlorella vulgaris Extract

A measured quantity (100 µL) of CVE was tested against multidrug-resistant (MDR) bacterial isolates using the agar well diffusion method. Briefly, 5-6 colonies (~1 mm in diameter) were picked from fresh agar plates and suspended in 5 mL of sterile saline followed by incubation at 37°C for 24 hours. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard. Sterile cotton swabs soaked in the inoculum were used to streak the surface of Mueller-Hinton agar (MHA) plates to prepare a uniform bacterial lawn and excess inoculum was removed by pressing the swab against the inner wall of the tube above the fluid level. Wells of 5 mm diameter were aseptically made in the agar using a cork borer and 100 µL of the algal extract was dispensed into each well in triplicate. Plates were incubated at 37°C for 24 hours and antibacterial activity was assessed by measuring the diameter of the inhibition zones around each well.

Turbidity Assay for Antimicrobial Activity of Chlorella vulgaris Extract

The antimicrobial activity of CVE against MDR bacterial isolates was evaluated using a turbidity assay. Fresh bacterial cultures were supplemented with optimized concentrations of the algal extract and incubated at 37°C for 24 hours with turbidity adjusted to an OD of 0.5. A 96-well ELISA plate was used to determine the minimum inhibitory concentration (MIC) with wells containing 130 µL, 32 µL and 20 µL of microalgae CVE inoculum. Based on the antibiotic resistance profiles from 100 clinical samples of which 10 MDR isolates (designated P1-P10) were selected for testing. For each isolate, triplicate control and experimental groups were prepared and varying concentrations of algal extract were added to the respective wells along with bacterial inoculum. Plates were incubated at 37°C for 24-48 hours and changes in bacterial growth were monitored by recording decreases in cell density in the presence or absence of microalgae CVE.

Biofilm Formation Assay

Biofilm formation was assessed using the microtiter plate assay with minor modifications. The bacterial isolates were first inoculated on nutrient agar and incubated at 37°C for 24 hours. Two to four colonies from each isolate were transferred into Brain Heart Infusion (BHI) broth and the turbidity was adjusted to match the 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/mL). A 200 µL suspension of each isolate was inoculated in triplicate into the wells of sterile flat-bottom 96-well polystyrene microtiter plates. Wells containing only BHI broth served as negative controls. The plates were incubated at 37°C for 24 hours to allow biofilm formation.

After incubation, the planktonic (non-adherent) cells were gently removed and each well was washed three times with 200 µL of sterile phosphate-buffered saline (PBS; pH 7.2) to remove unbound cells. The plates were then air-dried for 15 minutes at room temperature. The adherent biofilm was fixed with 200 µL of methanol per well for 15 minutes followed by air drying. Each well was then stained with 200 µL of 0.1% (w/v) crystal violet solution for 15 minutes at room temperature. Excess stain was removed by rinsing the wells three times with distilled water. The plates were air-dried again and the bound dye was solubilized with 200 µL of 95% ethanol for 15-20 minutes. Biofilm formation was quantified by measuring the optical density (OD) at 600 nm using a microplate reader.

Anti-Biofilm Activity of Microalgae CVE

The similar technique as described above was used for biofilm formation, except for the 3rd step in which suspension and algae extract were added in a 1:1 ratio. The biofilm formation inhibition % of microalgae CVE against each pathogen was calculated by mathematical equation.

$$\text{Inhibition of biofilm formation \%} = \frac{\text{OD control - OD after treatment}}{\text{OD control}} \times 100$$

Statistical Analysis

All collected data were entered into standardized proformas and analyzed using SPSS version 27 (IBM Corp., Armonk, NY, USA). Variables analyzed comprised patient demographics (name, age, sex) and clinical characteristics related to pus samples obtained from diabetic patients. Quantitative variables were presented as mean ± standard deviation (SD) whereas qualitative variables were summarized as frequencies and percentages. Associations between categorical variables were evaluated using the chi-square test and a p-value ≤ 0.05 was considered statistically significant.

RESULTS

This research study was to assess MDR bacterial growth from 100 diabetic pus samples against CVE. 10 MDR isolates from positive samples including burns, boils, trauma and abscesses were selected for further growth evaluation with the CVE.

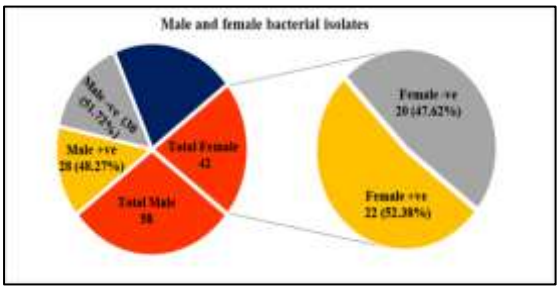

Fig.1 Distribution of positive and negative bacterial isolates

Fig.1 illustrates the distribution of bacterial isolates among male and female patients. Out of 100 samples 58 were from males and 42 from females. Among the male group 28 (48.27%) were culture-positive and 30 (51.72%) were culture-negative. In the female group, 22 (52.38%) were culture-positive and 20 (47.62%) were culture-negative. These results indicate that although the total number of male samples was higher (58 vs. 42), the proportion of positive isolates was slightly greater in females (52.38%) compared to males (48.27%). However in absolute numbers, males contributed more to the total positive isolates due to their larger sample size. Among the Gram-positive isolates *Staphylococcus aureus* was the most prevalent species (n = 20; 20%), followed by *Streptococcus pyogenes* (n = 18; 18%), *Staphylococcus epidermidis* (n = 6; 6%). *Enterococcus species* (n = 3; 3%) and *Clostridium perfringens* (n = 2; 2%). The Gram-negative isolates were predominantly *Escherichia coli* (n = 16; 16%), *Pseudomonas aeruginosa* (n = 12; 12%), and *Acinetobacter species* (n = 12; 12%), with smaller proportions of *Proteus mirabilis* (n = 6; 6%) and *Klebsiella pneumoniae* (n = 5; 5%).

These results demonstrate a higher prevalence of Gram-positive bacteria particularly *Staphylococcus aureus* while the current research also highlights the significant contribution of Gram-negative pathogens to diabetic wound infections.

Antimicrobial Assay

Antimicrobial activity of various antibiotics was tested against 10 MDR isolates. Microalgae CVE (50-400 µg/mL) was further evaluated for activity against *Staphylococcus aureus* and *Escherichia coli* (Table-1).

Table-1 Antimicrobial activity of *CVE*

Sr. No.	Concentration of *CVE* dispensed into each well in triplicate against *Staphylococcus aureus* and *Escherichia coli*	O.D (600nm)
1	Control (LB)	0.435
2	Control (NC)	0.445
3	Control (LB+Inoc)	0.553
4	Control (NC+Inoc)	0.592
5	CVE 50ug/mL	0.562
6	CVE 100ug/mL	0.504
7	CVE 150ug/mL	0.516
8	CVE 200ug/mL	0.509
9	CVE 250ug/mL	0.589
10	CVE 300ug/mL	0.681
11	CVE 350ug/mL	0.624
12	CVE 400ug/mL	0.675

CVE-Chlorella vulgaris extract, LB-Lysogeny broth. NC- Negative control, OD- Optical density, Inoc- Inoculum

The antimicrobial activity of microalgae CVE was evaluated at different concentrations and the results were recorded in terms of OD. Among the control groups, the lowest OD was observed in lysogeny broth (LB) medium alone (0.435) followed closely by the negative control (NC) at 0.445. Following the addition of bacterial inoculum OD values increased slightly reaching 0.553 for LB+Inoc and 0.592 for NC+Inoc

thereby confirming active bacterial growth in the inoculated controls. Treatment with algal extract demonstrated a concentration-dependent effect. The strongest inhibition was observed at 100 µg/mL (OD = 0.504) and 200 µg/mL (OD = 0.509) which were lower than the inoculum controls indicating effective antibacterial activity. However, higher concentrations (≥300 µg/mL) showed relatively higher OD values (0.624-0.681) suggesting reduced efficacy at very high doses. Overall the optimized inhibitory concentration of CVE was 100 µg/mL, which exhibited the most consistent bacterial growth reduction.

The antimicrobial activity of CVE and its combinations showed variable effects on bacterial growth (Table-2).

Table-2 Antimicrobial activity of microalgae *CVE* with variable amalgamations

Sr. No.	Concentration of *CVE*	O.D
1	LB	0.451
2	LB+Inoculum	0.625
3	KOH+Inoculum	0.331
4	KOH+Inoculm+LB	0.494
5	CVE+Inoculum+LB	0.540
6	AA+Inoculum	0.265
7	A.A+Inoculum+LB	0.762
8	MIX+Inoculum (100%)	0.661
9	MIX+LB+Inoculum (75%)	0.732
10	MIX+LB+Inoculum (50%)	0.492
11	MIX+LB+Inoculum (25%)	0.518
12	MIX+LB+Inoculum (12.5%)	0.907

CVE-Chlorella vulgaris extract, LB-Lysogeny broth, AA- Ascorbic acid, KOH-Potassium Hydroxide

The ascorbic acid (AA) exhibited the strongest inhibition (OD 0.265) followed by KOH (0.331) while CVE alone showed moderate activity (0.540). The presence of Lysogeny broth (LB) generally reduced antimicrobial efficacy with AA+LB even promoting growth (0.762). Mixture treatments revealed concentration-dependent responses where the 50% mixture (0.492) showed optimal inhibition while higher (100%, 75%) and lower (12.5%) concentrations were less effective or growth-promoting. These findings suggest that ascorbic acid (AA) and KOH are potent inhibitors whereas mixtures require optimized concentrations for maximal efficacy. The OD_{600} of the KOH+inoculum sample was 0.331 which was notably lower than the control (LB+inoculum). This reduction in turbidity indicates inhibited bacterial growth. Since potassium hydroxide (KOH) is a strong alkali its presence can significantly alter the pH of the growth medium creating an unfavorable environment for bacterial proliferation. Most bacteria including *Staphylococcus aureus* prefer near-neutral conditions (pH 6.5-7.5). Elevated alkalinity disrupts cell membrane integrity, enzyme activity and nutrient transport, ultimately suppressing metabolic processes. Therefore, the observed decrease in OD may not reflect only the antimicrobial potential of the test extract but could also result from the direct inhibitory effect of alkaline pH introduced by KOH.

Bacterial Growth in Liquid Culture Medium (Broth)

Bacterial isolates were grown in nutrient broth at 37°C for 24-48 hours and turbid cultures were selected for further processing. The CVE was then added and incubated for 24 hours after which bacterial growth inhibition was quantified using ELISA plate readings of OD for all 10 isolates.

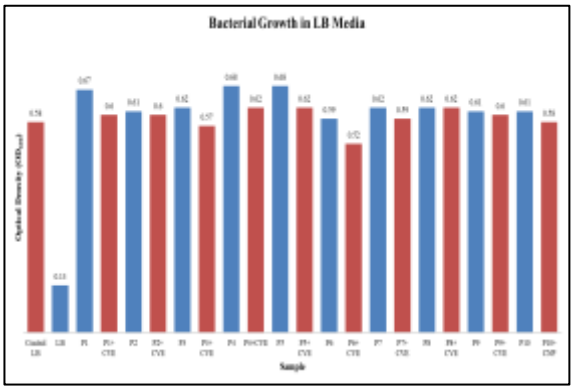

Fig. 2 Growth in broth solution of 10 MDR Bacterial Isolates

The Fig. 2 bar chart shows that microalgae CVE moderately inhibited growth of 10 MDR isolates in broth. Inhibition was isolate-dependent, with P1 and P6 showing notable reductions while P2 and P9 exhibited minimal changes. Overall these results suggest that CVE exerts a moderate inhibitory effect on different bacterial growth with the degree of inhibition being isolate dependent. For each isolate triplicate control and experimental groups were prepared.

The biofilm inhibition potential of CVE varied across the pus bacterial isolates obtained from diabetic patients (Table-3).

Table-3 Percentage of biofilm inhibition in pus isolates from diabetic patients treated with CVE

Sr. No.	Pus isolates	% Biofilm formation ability	% Inhibition at MIC with algal extract	MIC (µg/mL)	P-value
1	*Staphylococcus aureus*	39.44	29.25	200	0.011
2	*Staphylococcus epidermidis*	40.11	32.22	150	0.013
3	*Staphylococcus pyogenes*	29.14	42.12	100	0.009
4	*Escherichia coli*	43.22	35.44	250	0.012
5	*Klebsiella pneumoniae*	41.11	27.48	300	0.016
6	*Pseudomonas aerogenosa*	45.23	38.33	350	0.014
7	*Proteus mirabilis*	33.35	35.21	200	0.020
8	*Acenetobacter*	31.12	48.28	150	0.035
9	*Enterococcus species*	36.41	40.11	180	0.018
10	*Clostridium perfringens*	39.40	31.23	250	0.029

The findings in Table 3 demonstrate variable biofilm formation abilities and inhibition responses among the 10 pus isolates treated with CVE. Among all isolates, *Pseudomonas aeruginosa* exhibited the highest biofilm-forming potential (45.23%), followed by *Escherichia coli* (43.22%) and *Klebsiella pneumoniae* (41.11%). In contrast, *Acinetobacter species* and *Streptococcus pyogenes* demonstrated comparatively lower biofilm formation capacities (31.12% and 29.14% respectively). When exposed to CVE at their respective minimum inhibitory concentrations (MICs) a significant reduction in biofilm formation was observed in most isolates ($p<0.05$). The highest biofilm inhibition was recorded in *Acinetobacter species* (48.28%) and *Streptococcus pyogenes* (42.12%) indicating a strong anti-biofilm potential of algal metabolites against these pathogens. Conversely, *Klebsiella pneumoniae* and *Staphylococcus aureus* exhibited lower inhibition levels (27.48% and 29.25% respectively) suggesting greater resistance possibly due to protective capsular polysaccharides or enzyme-mediated tolerance.

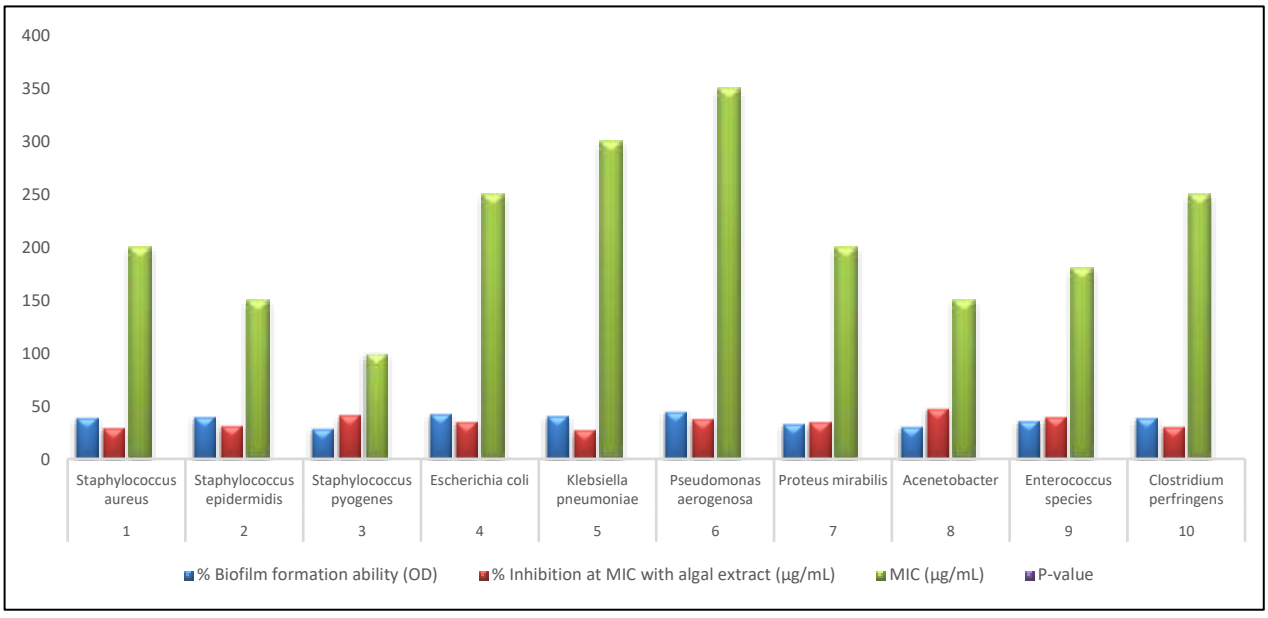

Fig. 3 Biofilm inhibition percentage (%) at varying MIC levels of CVE against pus isolates from diabetic patients

Fig 3 illustrates the biofilm formation ability and percentage inhibition at MIC levels for the 10 pus isolates treated with *CVE.* The bar chart shows that *Pseudomonas aeruginosa* and *Escherichia coli* had the highest biofilm formation capacities (45.23% and 43.22%, respectively) whereas *Streptococcus pyogenes* and *Acinetobacter*

species demonstrated lower biofilm formation (29.14% and 31.12%).

Upon exposure to CVE at their MIC levels a marked decrease in biofilm biomass was recorded across all isolates. The highest inhibition was observed for *Acinetobacter species* (48.28%) and *Streptococcus pyogenes* (42.12%), indicating strong susceptibility to algal metabolites while *Klebsiella pneumoniae* and *Staphylococcus aureus* showed comparatively weaker inhibition (27.48% and 29.25%). The MIC range of 100-350 µg/mL reflected concentration-dependent activity where isolates with lower MICs displayed greater sensitivity and biofilm suppression.

DISCUSSION

Chlorella vulgaris is a microalgae widely used in Japan as a nutritional supplement[11]. In addition, extracts of *Chlorella vulgaris* contain bioactive compounds that exhibit antioxidant, anti-inflammatory, antitumor and antibacterial activities[12]. Microalgae CVE derivatives are a source of alternative treatment therapies such as hexane extract which has powerful anti-bacterial and anti-biofilm properties against isolates from burn and surgical patients[13]. The strong antimicrobial efficacy of microalgae CVE is attributed to its bioactive contents including alkaloids, terpenoids, phenols and high levels of fatty acids and lipids. These compounds were identified using the GC-MS technique[14]. CVE exerts its antimicrobial activity through a multifaceted molecular mechanism involving membrane disruption, oxidative damage, enzymatic inhibition, quorum-sensing interference and biofilm suppression[15]. These mechanisms collectively explain its strong inhibitory potential against MDR bacterial isolates observed in the current research study.

Among isolates, *Staphylococcus aureus* (25%) was predominant with high resistance in *Streptococcus pyogenes* (P4, 90%). Gram-negative isolates were largely resistant to ciprofloxacin/amikacin but sensitive to carbapenems partly aligning with previous diabetic infection research studies[16,17]. These findings align with previous studies showing *Staphylococcus aureus* predominance and Gram-negative resistance to ciprofloxacin/amikacin yet differ by the unusually high resistance in *Streptococcus pyogenes* possibly due to regional antibiotic pressure or emerging resistance mechanisms.

The current research study results indicated that the Kirby-Bauer technique was not effective in inhibiting the growth or killing the isolated bacteria regarding microalgae CVE. It was ineffective with CVE likely because the extract's bioactive compounds diffuse poorly through agar resulting in limited zone formation. Additionally the crude algal extract may require higher concentrations or specific solvents for optimal antimicrobial activity which the disc diffusion method does not adequately support. This finding contrasts with the results of El-a research regarding the effectiveness of this method[18]. The observed dissimilarity in microbiological assays could be attributed to various factors such as inoculum concentration, agar media composition, pH, solubility, sample size, humidity and exposure time, all of which may influence assay outcomes[18,19].

The results of the turbidity method using the microplate ELISA kit showed that the bacterial isolates displayed reduced cell densities as compared to the control without algal extract. The recorded results are supportive of previous findings[20]. The CVE showed moderate inhibition against MDR bacterial isolates from diabetic pus with variable susceptibility among strains. These results align with previous reports[20] but differ slightly from Morowvat (2023) possibly due to differences in bacterial species, extraction methods or experimental protocols, highlighting the need for standardized antimicrobial evaluation[21].

According to previous research studies, CVE has long been recognized for its antibiotic properties acting as a biomaterial to eradicate pathogenic microbes, aid in wound healing and contribute to pharmaceutical applications as described by previous studies[22]. The CVE was effective against biofilms formed by the isolated bacterial pathogens which is consistent with previously reported studies[23]. Moreover, CVE has been identified as a promising anti-biofilm agent.[24]. Its antibacterial activity is also valuable against MDR bacteria[25,26].

CONCLUSION

The current research study demonstrated that CVE exhibits measurable antimicrobial and anti-biofilm activity against MDR bacterial isolates from diabetic patients. Significant activity CVE may serve as an effective topical option for managing pyogenic wounds and offers promising applications in the pharmaceutical industry. Nevertheless, further investigations including purification of active metabolites, mechanistic studies and in vivo validation are essential to fully establish its therapeutic potential.

Acknowledgments

The authors would like to acknowledge the support of the Department of Pathology, Allama Iqbal Medical College, Lahore and the Faculty of Science and Technology (FOST) University of Central Punjab for providing the facilities to conduct this research.

Author Contributions

Ahmad Naeem Sajed conceived and designed the study. **Muhammad Waseem** and **Muhammad Ahsan Murtaza** collected and analyzed the data. **Muhammad Tayyab** assisted in laboratory experiments and data interpretation**. Mohsin Gulzar Barq** and **Abdul Nafeh** contributed to manuscript writing and critical revision. All authors read and approved the final manuscript.

Ethical Approval

The current research study received approval from the Ethical Review Board (IRB-SD-27 12-05 2023) of Allama Iqbal Medical College/Jinnah Hospital, Lahore.

Grant Support and Funding Disclosure

None.

Conflict of Interests

None.

REFERENCES

1. Demir S, Nawroth PP, Herzig S, Ekim Üstünel B. Emerging targets in type 2 diabetes and diabetic complications. Advanced Science. 2021 Sep;8(18):2100275. **DOI:** https://doi.org/10.1002/advs.202100275
2. Inamadar AC, Palit A. Cutaneous Bacterial Infections. In: Atlas of Dermatology, Dermatopathology and Venereology: Cutaneous Infectious and Neoplastic Conditions and Procedural Dermatology. Cham: Springer International Publishing; 2021 Oct 5. p. 1-22. **DOI:** https://doi.org/10.1007/978-3-319-53805-1_72
3. Linz MS, Mattappallil A, Finkel D, Parker D. Clinical impact of Staphylococcus aureus skin and soft tissue infections. Antibiotics. 2023 Mar 11;12(3):557. **DOI:** https://doi.org/10.3390/antibiotics12030557
4. Donnelly J, Collins A. Wound management, tissue viability and infection. Orthopaedic and Trauma Nursing: An Evidence-based Approach to Musculoskeletal Care. 2023 Feb 10:140-56. **DOI:** https://doi.org/10.1002/9781119833413.ch12
5. Heffernan AJ, Lim SM, Lipman J, Roberts JA. A personalised approach to antibiotic pharmacokinetics and pharmacodynamics in critically ill patients. Anaesthesia Critical Care & Pain Medicine. 2021 Dec 1;40(6):100970. **DOI:** https://doi.org/10.1016/j.accpm.2021.100970
6. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DH, Hozzein WN, Batiha GE, et al. Methicillin-Resistant Staphylococcus aureus (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infection and Drug Resistance. 2020 Sep 22:3255-65. **DOI:** https://doi.org/10.2147/IDR.S272733
7. Urban-Chmiel R, Marek A, Stępien-Pysniak D, Wieczorek K, Dec M, Nowaczek A, et al. Antibiotic resistance in bacteria-A review. Antibiotics. 2022 Aug 9;11(8):1079. **DOI:** https://doi.org/10.3390/antibiotics11081079
8. Alara JA, Alara OR. An overview of the global alarming increase of multiple drug resistant: a major challenge in clinical diagnosis. Infectious Disorders-Drug Targets. 2024 May 1;24(3):26-42. **DOI:** https://doi.org/10.2174/1871526523666230725103902
9. Ilieva Y, Zaharieva MM, Kroumov AD, Najdenski H. Antimicrobial and ecological potential of Chlorellaceae and Scenedesmaceae with a focus on wastewater treatment and industry. Fermentation. 2024 Jun 28;10(7):341. **DOI:** https://doi.org/10.3390/fermentation10070341
10. Yin D, Guo Y, Han R, Yang Y, Zhu D, Hu F. A modified Kirby-Bauer disc diffusion (mKB) method for accurately testing tigecycline susceptibility: a nation-wide multicenter comparative study. Journal of Medical Microbiology. 2023 Aug 8;72(8):001671. **DOI:** https://doi.org/10.1099/jmm.0.001671
11. Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. European Journal of Microbiology and Immunology. 2024 May 14;14(2):97-115. **DOI:** https://doi.org/10.1556/1886.2024.000355
12. Kaur J, Nobile CJ. Antifungal drug-resistance mechanisms in Candida biofilms. Current Opinion in Microbiology. 2023 Feb 1;71:102237. **DOI:** https://doi.org/10.1016/j.mib.2022.102237
13. Thajuddin N. Development of wound dressing film using methanolic extracts of freshwater microalgae and determining its wound healing ability. **DOI:** https://doi.org/10.25081/cb.2021.v12.6986
14. Nesrullah Z, Al-Rubaiee G, Zaki N. Evaluation of the extract of microalgae and its fatty acids on Candida spp. isolated from renal impairment patients. IUJAS. 2023;7(1):252-71. **DOI:** https://doi.org/10.52865/RNLN4662
15. Zuorro A, Lavecchia R, Contreras-Ropero JE, Martínez JB, Barajas-Ferreira C, Barajas-Solano AF. Natural antimicrobial agents from algae: Current advances and future directions. International Journal of Molecular Sciences. 2024 Nov 4;25(21):11826. **DOI:** https://doi.org/10.3390/ijms252111826

16. Iddrisu AK, Owusu G, Doe SK, Yeboah AA, Agyapong J, Yankey N. Uropathogens and their antibiotic susceptibility patterns among diabetic patients at st. john of god hospital, duayaw nkwanta, Ghana: a cross-sectional study. Health Science Reports. 2024 Sep;7(9):e70072.
DOI: https://doi.org/10.1002/hsr2.70072
17. Ahmed AQ, Mohammed NJ, Zefenkey ZF, Mamand SF, Hassannejad S, Hassan AO, et al. Investigate Freshwater Algae Extract's Efficacy in Treating Diabetes Ulcers and Its Anti-Staphylococcal Properties. Reports of Biochemistry & Molecular Biology. 2024 Apr;13(1):114.
DOI: https://doi.org/10.61186/rbmb.13.1.114
18. El-Salamouni NS, Gowayed MA, Seiffein NL, Abdel-Moneim RA, Kamel MA, Labib GS. Valsartan solid lipid nanoparticles integrated hydrogel: A challenging repurposed use in the treatment of diabetic foot ulcer, in-vitro/in-vivo experimental study. International Journal of Pharmaceutics. 2021 Jan 5;592:120091.
DOI: https://doi.org/10.1016/j.ijpharm.2020.120091
19. Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. European Journal of Microbiology and Immunology. 2024 May 14;14(2):97-115.
DOI: https://doi.org/10.1556/1886.2024.00035
20. Hussien FH, AL-Ammar MH. Molecular study of some pathogenic bacteria associated with the diabetes patients. International Journal of Health Sciences. 2022;6(S4):6829-38.
DOI: https://doi.org/10.53730/ijhs.v6nS4.10309
21. Morowvat MH, Kazemi K, Jaberi MA, Amini A, Gholami A. Biosynthesis and antimicrobial evaluation of zinc oxide nanoparticles using Chlorella vulgaris biomass against multidrug-resistant pathogens. Materials. 2023 Jan 15;16(2):842.
DOI: https://doi.org/10.3390/ma16020842
22. Sultan YY, Marrez DA. Isolation and purification of antifungal compounds from the green microalga Chlorella vulgaris. Journal of Applied Biotechnology Reports. 2022 Jun 1;9(2):603-13.
DOI: https://doi.org/10.30491/jabr.2021.302307.1438
23. Christabel PF, Hernando MV, Sutanto CA, Parisihni K. Exploration of Chlorella spp. as antibacterial to Aggregatibacter actinomycetemcomitans biofilm. In: IOP Conference Series: Earth and Environmental Science. Vol. 217, No. 1. IOP Publishing; 2018 Dec 1. p. 012040.
DOI: https://doi.org/10.1088/1755-1315/217/1/012040
24. Li SF, Fanesi A, Martin T, Lopes F. Biomass production and physiology of Chlorella vulgaris during the early stages of immobilized state are affected by light intensity and inoculum cell density. Algal Research. 2021 Nov 1;59:102453.
DOI: https://doi.org/10.1016/j.algal.2021.102453
25. Dvoretsky D, Dvoretsky S, Temnov M, Markin I, Akulinin E, Golubyatnikov O, et al. Experimental research into the antibiotic properties of Chlorella vulgaris algal exometabolites. Chemical Engineering Transactions. 2019 Apr 1;74.
DOI: https://doi.org/10.3303/CET1974239
26. Ricky R, Chiampo F, Shanthakumar S. Efficacy of Ciprofloxacin and Amoxicillin Removal and the Effect on the Biochemical Composition of Chlorella vulgaris. Bioengineering. 2022 Mar 24;9(4):134.
DOI: https://doi.org/10.3390/bioengineering9040134