

## The antimicrobial effects of *Chlorella vulgaris* extracts on pathogenic bacteria isolated from burn patients

Marrwa Mohammed Majeed<sup>1</sup>, Ahmed Shaker Alashoor<sup>2</sup>, Badr Alaoui-Sosse<sup>3</sup>

<sup>1,2</sup>Thi-Qar Health Office, University of Thi-Qar College of Science

<sup>3</sup>Laboratoire Chrono-Environnement - UMR 6249, Université de Bourgogne Franche-Comté, 16, route de Gray, 25000, Besançon, France  
Email: Marrwa70m@gmail.com

### Abstract

The use of dried algal biomass and algal derived biologically active compounds as pharmaceuticals has received much attention recently. The aims of this study are to isolate common pathogenic bacteria infected burn patients, and study of the biological effect of the extracted organic materials on the bacteria isolated from burn patients. A total of 100 swab samples were collected from burn patients at Al-Imam Al-Hussein Teaching Hospital and Special Burn Clinics in Thi-Qar province/Southern of Iraq. Selected samples were labeled and cultivated on blood agar and MacConkey. The API-20 system was used to diagnose isolated bacteria *S. aureus*. The current study indicated the highly activity of hexane and ethanol extract with concentration 100% extracted from *Chlorella vulgaris* has highly activity against *Staphylococcus aureus*, *Pseudomonas Aeruginosa*. It also showed that the most infectious bacteria was isolated from burn patients was *P. aeruginosa* 32.7%, followed *E. coli* and *S. aureus* 19.2%. The most common bacteria isolated from burns were *P. aeruginosa*, *E. coli* and *S. aureus*, while the lowest infectious bacteria isolated were *Enterococcus*. Depending on the present study, the hexane and ethanol - extracted from *C. vulgaris* solution has highly activity against *S. aureus* and *P. aeruginosa* isolated from burns.

**Keywords:** antimicrobial effects, *Chlorella vulgaris*, pathogenic bacteria, burn patients

### Introduction

Burn injuries were the most sources for patient morbidity and mortality followed by bacterial infection, as outlined by the World Health Organization (WHO), the burden of injury falls predominantly on people living in low and middle income countries over 95% of the 300,000 annual deaths from fires occur in these countries [1]. The severity of burns was divided according to the depth of the tissues affected, in the case of burns to the skin this is the layers of cells in the skin, epidermal burns (known first degree of burns) are confined to the epidermis, are not usually significant injuries and heal rapidly and spontaneously. Partial thickness burns (known second degree of burns) involve varying amounts of the dermis (skin) [2]. Partial-thickness burns are divided into superficial and deep partial thickness wounds, superficial partial thickness burns extend into the papillary or superficial upper layer of the dermis, whilst deep partial-thickness burns extend downward into the reticular layer of the dermis. Full-thickness burns (known third degree burns) extend through all the layers of the skin [3]. Burn infection is a major challenge in burn care and is the most common cause of mortality after burn injury; pathogens have evolved overtime in line with innovations and antibiotic use [4]. Bacterial organisms causing burn wound infection can be classified into two groups, gram negative and gram positive [5]. Gram negative bacteria cause most burn wound infections, with similar incidence, prevalence and pathogens, burn wound infection by *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherchia coli* and the gram positive organism *Staphylococcus aureus* are independent predictors of mortality, *S. aureus* is the major cause of gram positive burn wound infections globally and a common cause of septicemia [6]. Methicillin resistant *S. aureus* (MRSA) is now the major pathogen in some burn centers and vancomycin resistant enterococci (VRE) although not as common, appears to be highly virulent [7]. The biologically active compounds extract from microalgae were best sources of various such as sterols, polysaccharides, phenolic compounds, carotenoids and fatty acids, the use of dried algal biomass and algal derived biologically active compounds as pharmaceuticals has received much attention recently [8]. Polysaccharides from microalgae are potent, anti-inflammatory, immunomodulatory, hypo-cholesterolemic,

hypo-lipidomic and hypoglycemic agents [9]. The aims of this study are to isolate common pathogenic bacteria infected burn patients, and study of the biological effect of the extracted organic materials on the bacteria isolated from burn patients.

## **Material and Methods**

### **Patients and methods**

A total of 100 swab samples were collected from burn patients at Al-Imam Al-Hussein Teaching Hospital and Special Burn Clinics in Thi-Qar province/Southern of Iraq, during the period from 15 August 2020 to 15 December 2020, the burn patients' age range from 1.5 to 70 years from both gender. Swabs taken from burn patients are transported directly to the laboratory by conveyors.

### **Culturing of Samples**

Selected samples were labeled and cultivated on blood agar and MacConkey agar for swab cultivated on MacConkey agar only incubated overnight at 37°C in incubator in both aerobic and anaerobic conditions. For *E. coli* and *K. pneumonia* suspected from swab sample make subculture on blood agar and MacConkey agar. *Proteus mirabilis* grow on the blood agar plate in successive waves to form a thin filmy layer of concentric circles swarming, *P. mirabilis* do not swarm in the MacConkey agar medium and form smooth, pale or colorless colonies. The colonies of *P. aeruginosa* are colorless due to the lack of lactose fermentation which is of great importance in differentiating *P. aeruginosa* from other bacteria present in the specimen, especially from gram-positive bacteria, *S. aureus* on blood agar, growth occurs abundantly within 18 to 24 hours. Round, raised, opaque, yellow to golden yellow colonies of 1-2mm in diameter are seen with or without beta hemolysis. As explained in Frol and Iurp, (2012), by take D.W. by loop full and a single colony from culture was taken by a loop placed on clean slide and spread, then wait to dry and fixing by heat, staining by gram stains and examined the bacterial cell under microscope (oil immersion).

### **Analytical Profile Index**

The API-20 system was used to diagnose isolated bacteria *S. aureus* and Enterobacteriaceae; this system consists of a container bar containing 20 biochemical reactions distributed in single micro tubes.

### **Preparation of Extracts**

A twenty grams of dried powder of *C. vulgaris* previously prepared in college of science was extracted once with 200 ml of hexane, and other with 200 ml of ethanol and by Soxhlet continuous extraction, the solution was filtered by using whatmann No.13 filter paper then the filtrate was concentrated under reduced pressure on a rotary evaporator at 50°C and dried at 25°C, the extract were collecting in sterilized glass tubes until use.

## **Results**

### **Identification of Bacteria from Burn Infectious Patient**

The current results indicated that the most infectious bacteria was isolated from burn patients was *P. aeruginosa* 14.2%, followed *E. coli* and *S. aureus* 8.33%, while the lowest infectious bacteria isolated were *P. mirabilis* 3.33% (as shown in figure 3-1).

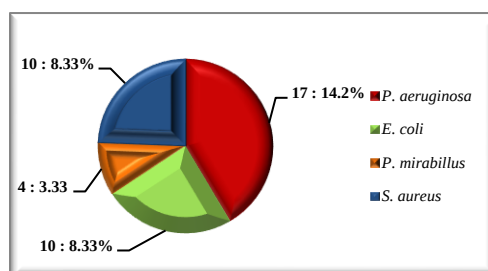


Figure 3-1: Identification of pathogenic bacteria in burn patients

### Organic Compounds Extract from *Chlorella vulgaris* by Ethanol Extract

The organic compounds were extracted from *C. vulgaris* by GC-Mass technology, the results indicated that the ethanol extract contained eight organic compounds, each of them took a specific distance within the time of retention, the organic compound that have high effectiveness on the isolated bacteria and yeast are those that have a higher distance from other organic compounds.

Table 3-1: Organic compound Extract from *C. vulgaris* by ethanol extract

| Peak | R. Time | Common Name                              | Peak Report TIC Area% |
|------|---------|------------------------------------------|-----------------------|
| 1-   | 6.737   | -Methoxy-N-methylacetamide               | 8.91                  |
| 2-   | 11.999  | Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-  | 44.49                 |
| 3-   | 19.284  | Isobutyl nitrite                         | 13.61                 |
| 4-   | 19.537  | Carbonic acid, cyclic ethylene ester     | 0.58                  |
| 5-   | 20.783  | Propanoic acid, 2-methyl-, anhydride     | 5.70                  |
| 6-   | 21.014  | 5,10-Pentadecadien-1-ol, (Z,Z)-          | 4.90                  |
| 7-   | 21.086  | Pyrrole                                  | 2.54                  |
| 8-   | 24.612  | Phthalic acid, 4-cyanophenyl nonyl ester | 19.27                 |

### Organic Compounds Extract from *Chlorella vulgaris* by Hexane Extract

The organic compounds were extracted from *C. vulgaris* algae in laboratories of the Environmental Research Center/Ministry of Science and Technology by GC-Mass technology, the results indicated that the hexane extract contained only two organic compounds, each of them took a specific distance within the time of retention. The organic compound that has high effectiveness on the isolated bacteria and yeast are those that have a higher distance from other organic compounds.

Table 3-2: Organic compound Extract from *C. vulgaris* by hexane extract

| Peak | R. Time | Common Name                                      | Peak Report TIC Area% |
|------|---------|--------------------------------------------------|-----------------------|
| 1-   | 19.267  | Butanoic acid, 2-hydroxy-2-methyl-, methyl ester | 90.02                 |
| 2-   | 20.775  | Furan, 2-(dichloromethyl)-tetrahydro-            | 9.98                  |

### Active Organic compounds Extracts from *C. vulgaris* by Hexane and Ethanol Extract

The table represents the mass spectrometry of organic compounds that formed a high percentage and isolated from the *C. vulgaris* by both hexane and ethanol extract which is

believed to have biological and antimicrobial activity against isolated pathogenic bacteria and yeast under study.

Table 3-3: Active Organic compounds Extracts from *C. vulgaris*

| Peak | R. Time | P. Area | R. Comp Name<br><i>C. Vulgaris</i> Hexane        | M. Weight | Chemical Structure     |
|------|---------|---------|--------------------------------------------------|-----------|------------------------|
| 1-   | 19.267  | 90.02   | Butanoic acid, 2-hydroxy-2-methyl-, methyl ester | 132       | <chem>C6H12O3</chem>   |
| Peak | R. Time | P. Area | R. Comp Name<br><i>C. Vulgaris</i> Ethanol       | M. Weight | Chemical Structure     |
| 2    | 11.999  | 44.49   | Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-          | 164       | <chem>C10H12O2</chem>  |
| 8    | 24.612  | 19.27   | Phthalic acid, 4-cyanophenyl nonyl ester         | 393       | <chem>C24H27NO4</chem> |

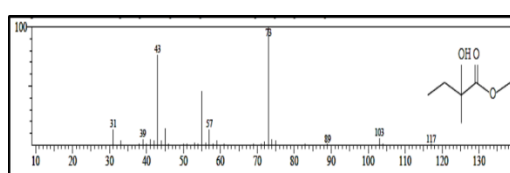


Figure 3-2: The mass spectrum of the chemical Butanoic acid, 2-hydroxy-2-methyl-, methyl ester isolated from the hexane extract

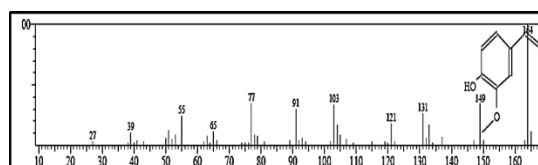


Figure 3-3: The mass spectrum of the chemical Phenol, 2-methoxy-4-(1-propenyl)-, (Z)- isolated by ethanol extract

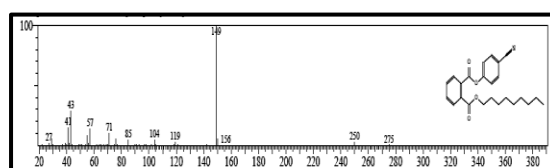


Figure 3-4: The mass spectrum of the chemical Phthalic acid, 4-cyanophenyl nonyl ester isolated by ethanol extract

### Activity of *C. vulgaris* Hexane Extract Against isolated bacteria

The current study indicated the highly activity of extract with concentration 100% extracted from *C. vulgaris* has highly activity against *S. aureus*, *P. aeruginosa*, and the lowest activity showed against *P. mirabillis*. Also the antibacterial activity showed increased with increase concentration of extract (as shown in table 1).

Table 3-4: Activity of *C. vulgaris* hexane extract against isolated bacteria

| Pathogens CON |            | <i>P. aeruginosa</i>                       | <i>S. aureus</i> | <i>K. pneumonia</i> | <i>P. mirabillis</i> |
|---------------|------------|--------------------------------------------|------------------|---------------------|----------------------|
|               |            | Inhibition Zone/ mm <sup>2</sup> Mean + SD |                  |                     |                      |
| 100%          | Case No. 5 | 30.0 ± 0.8                                 | 41.5 ± 1.3       | 24.1 ± 2.7          | 21.1 ± 0.7           |

|          |  |            |            |            |            |
|----------|--|------------|------------|------------|------------|
| 75%      |  | 22.1 ± 1.7 | 22.6 ± 1.9 | 21.1 ± 0.7 | 17.8 ± 1.1 |
| 50%      |  | 19.1 ± 1.7 | 17.8 ± 1.3 | 16.8 ± 2.0 | 11.0 ± 1.5 |
| 25%      |  | 13.1 ± 1.7 | 9.50 ± 1.0 | 11.1 ± 1.7 | 8.66 ± 0.8 |
| P. Value |  | < 0.01     | < 0.01     | < 0.01     | < 0.01     |
| LSD      |  | 1.87       | 1.76       | 2.36       | 1.34       |

### Activity of *C. vulgaris* Ethanol Extract against Isolated Bacteria

The current study indicated the high activity of extract with concentration 100% extracted from *C. vulgaris* has highly activity against *S. aureus*, *P. aeruginosa*, and the lowest activity showed against *P. mirabilis*. Also the antibacterial activity showed increased with increase concentration of extract (as shown in table 2).

Table 3-5: Activity of *C. vulgaris* ethanol extract against isolated bacteria

| Pathogens |              | <i>P. aeruginosa</i>                       | <i>S. aureus</i> | <i>K. pneumonia</i> | <i>P. mirabilis</i> |
|-----------|--------------|--------------------------------------------|------------------|---------------------|---------------------|
| CON       |              | Inhibition Zone/ mm <sup>2</sup> Mean + SD |                  |                     |                     |
| 100%      | Cases No.= 6 | 25.5 ± 1.3                                 | 34.6 ± 1.9       | 22.1 ± 2.1          | 17.6 ± 1.2          |
| 75%       |              | 19.0 ± 1.8                                 | 17.6 ± 1.5       | 19.6 ± 1.2          | 16.0 ± 0.9          |
| 50%       |              | 16.0 ± 1.5                                 | 15.0 ± 1.5       | 15.6 ± 2.2          | 9.16 ± 0.7          |
| 25%       |              | 11.1 ± 1.7                                 | 6.00 ± 1.5       | 10.0 ± 1.5          | 6.16 ± 0.7          |
| P. Value  |              | < 0.01                                     | < 0.01           | < 0.01              | < 0.01              |
| LSD       |              | 1.98                                       | 1.99             | 2.21                | 1.11                |

### Discussion

Worldwide, the burns infection was responsible for more than 300,000 deaths cases annually; the infection is a major cause of morbidity and mortality in these burn patients. Early diagnosis and treatment of this microbial infection was improves outcome. Toward this end it is necessary to identify the institutions flora and organisms that most frequently produces infection [10] (Ramirez-Blanco *et al.*, 2017). The current study recorded 52% of patient with burn infects with pathogenic bacteria and *Candida* spp, and 48% have not infection where our study recorded that the highest percentage of isolated bacteria was *P. aeruginosa* 14.2%, *S. aureus* and *E. coli* 8.3%, *K. pneumoniae* and mixed bacterial infection 3.3%. Previous stud performed by Abduljabbar *et al.*, (2020), in Al-Najaf province, they study prevalence of aerobic pathogenic bacteria isolated from patients with burn infection, and concluded 57.5% of patient infected with both gram positive and negative bacteria and the infection rate in their study was similar with the infection rate in the current study , and there was a high prevalence of multi-drug resistant bacteria in burn infection was *P. aeruginosa* 27.6%, followed by *S. aureus* 20.7% and among this finding percentage 76.2% were mixed bacterial infection and the other bacterial types consist 9.2%, the isolated bacteria was incompatible with the isolated bacteria in their study in terms of the number and type of isolated bacteria.

### Chemical Reagent of the Extract

Chemical reagents are of great importance to detect the active chemical compounds present in the ethanol and hexane extract, especially the secondary metabolites, because of their high biological efficacy [11]. The current study indicated *C. vulgaris* ethanol extract had alkaloids, phenol and soaps, and hexane extract contain alkaloids, amino acids, carbohydrates, phenols, tannins and flavonoids, the study also recorded alkaloids, amino acids, tannins, glycosides and flavonoids not detected in both ethanol and hexane extracts, while glycosides only not indicated in both ethanol and hexane extracts. The study of [12], they study supplements in both *S. platensis* and *C. vulgaris* and separation and identified both algae contain carotenoids, chlorophyll in dietary supplements and chemical compound important in medical and nutritional application. Due to the beneficial properties on human health and the nutritional supplements used in the animal husbandry fields of these compounds, increasing numbers of carotenoid supplements have appeared on the market in recent years. Therefore, methods of rapid and accurate identification of these compounds in nutritional supplements are urgently required in order to ensure their quality and safety, and to protect the interests of consumers [13].

### Gas Chromatography and Antibacterial Activity

One of the most important techniques used in the detection and identification of secondary metabolic compounds of hexane and ethanol from living organisms such as cyanobacteria and green algae for isolated and identification of chemical compound have medical application for pathogenic controlling, and in addition this compounds can easily degrades by other living organism and this was confirmed by a study [14]. Chemicals compounds were isolated and their effectiveness studied on pathogenic bacteria isolated from burn patients, Butanoic acid, 2-hydroxy-2-methyl-, methyl ester from *C. vulgaris* hexane extract, Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-, Isobutyl nitrite and Phthalic acid, 4-cyanophenyl nonyl ester from *C. vulgaris* ethanol extract due to biological activities on pathogenic microbes, the activity of *C. vulgaris* hexane and ethanol extract were showed among four concentration used the activity of both hexane and ethanol extracts increased with increasing concentration. The previous study of [15] they extract *C. vulgaris* by acetone, ethanol and chloroform and tests the against pathogenic bacteria and concluded the highest activity of acetone extract against *Bacillus* spp and the lowest against *E. coli*, the ethanol extract exhibited maximum activity against *Klebsiella* spp and the lowest activity against *Pseudomonas* spp, while the chloroform extract exhibited their maximum activity against *Bacillus* spp and the minimum activity was against *Klebsiella* spp. Also the study of Uma *et al.* [16], they study the antimicrobial activity of *C. vulgaris* were extracted by acetone, methanolic, ethanolic and DMSO, and the extracts were assayed against multi-drugs resistant bacteria *K. pneumoniae*, *Pseudomonas* spp, *V. cholerae*, *S. pyogenes* and *E. coli*, and their study concluded the *Pseudomonas* spp was most bacteria effected by extracts of acetone, methanolic and ethanolic, while the DMSO extract exhibited maximum activity against *K. pneumoniae*, their results also noted the lowest response to extract activity was *V. cholera*. A study performed by Ara *et al.* [17], in Saudi Arabia Kingdom, they study activity of chemical compound detected from seven different families medical plant against pathogenic bacteria and yeasts isolated from clinical samples, and recorded butanoic acid, 4-ethyl-2-hydroxycyclopent-2-en-1-one, 2-methoxy-4-vinylphenol, and cyclobutano had antimicrobial activity against *P. aeruginosa*, *E. coli*, *S. aureus*, *B. subtilis* and *C. albicans*, while the study also concluded that ethanol extracts from 70% of the plants were toxic to cell and only one of species of *Combretum duarteanum* recorded has antimicrobial activity, the toxicity of extracts from *Arthemus sativa*, which is known to have antimicrobial activity, the result also indicated the acetone extract of butanoic acid was cidal for both *E. coli*, *S. aureus* and *C. albicans* while

static for *P. aeruginosa*. The antimicrobial activity of the n-butanol and Isobutyl nitrite isolated from *C. vulgaris* fraction could be due to the presence of monoterpene, diester of phthalic acid, saturated fatty acids and monocyclic phenolic compounds [18]. A study of Dubey *et al.* [19] in India, they isolated chemical compound from *Woodfordia fruticosa* and recorded among chemical isolated Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-, Isobutyl nitrite, had antibacterial, antifungal, anthelmintic, antiseptic, anti-inflammatory, antihemolytic anticancer,, antioxidant, antidiabetic, antiparasitic, and wound healing activities and their activity were performed against gram positive and gram negative multi drug resistant bacteria that resistant for different class of antibiotic, and the isolated chemical compounds were inhibit growth of bacteria, also they study their toxicity and concluded both Phenol, 2-methoxy-4-(1-propenyl)-, (Z)- and Isobutyl nitrite not toxic when their mix with both hole blood and when mixed with lymphocyte, also they tested for minimum inhibiter and minimum bactericidal activity, and indicated. The phyto-compounds such as alkaloids, glycosides, terpenoids, steroids, saponins, and tannins were present in the algal extract and these compounds had contributed to the recorded control of pathogenic bacteria and fungi, this extracts could be used as a part of an integrative treatment of the pathogen as antimicrobial agent of non- microbial infection origin along with mainstream antimicrobial drugs [11]; Roy *et al.* [20].

## Conclusion

The most common bacteria isolated from burns were: *P. aeruginosa*, *E. coli*, *S. aureus*, while the lowest infectious bacteria isolated were *P. mirabilis*. Depending on the present study, the hexane and ethanol- extracted from *C. vulgaris* solution has highly activity against *S. aureus*, *P. aeruginosa* isolated from burns.

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