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Research Article

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Analysis of value–added biochemical compounds and antimicrobial activity of green algae *Chlorella vulgaris*

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ABSTRACT

Chlorella vulgaris is widely cultivated micro algae found in water bodies as it is rich in nutrients and its component of phytoplankton. C.vulgaris is useful in biomass production in commercial level as it contains proteins and minerals. In this study the value added biochemical compounds in C.vulgaris were analyzed from different cultures. The amount of total carotenoids, protein, vitamin, pigments and phenolic compounds were examined. C.vulgaris extracts possessed great potential antibacterial activities against four bacterial strains with inhibition zones ranged 8-15mm and MICs values ranges from 25-40ųg/mL.Thus, it could be suggested that the C.vulgaris is useful bio-system for production of biochemical compounds possess an antimicrobial principles.

Key Words: *Chlorella vulgaris*, Biochemical compounds, Culture media, Organic extract.

INTRODUCTION

Microalgae are the rich source of proteins and other nutrients, similar to higher plants. Microalgae play important role as primary producers for various consumers and rich source of protein, carbohydrate and especially essential fatty acids. *Chlorella vulgaris* is a globular green algae and cosmopolitan in occurrence [1]. The genus of chlorella includes a varied range of species with high temperature tolerance to growing autotrophically in an inorganic medium [2].

Since 1950 production of *C.vulgaris* was carried out in commercial scale in countries like Japan and Taiwan. Among the various microalgae that have been explored for their suitability for commercial potential, *chlorella* species are major type that have been used successfully to produced high concentration of valuable compounds. *C.vulgaris* is highly valued for its protein content as it can be used for its potential biomass [3]. The present study was made an attempt to analyze the biochemical compounds from *C.vulgaris*, which has a potential nutrient value. In addition, the organic extracts of algal cultures were assessing for their antimicrobial activities.

EXPERIMENTAL SECTION

Water sample was collected from ponds and microalgae were isolated using Bold Basal Medium(BBM).The cultures were grown at 24±1°C in a thermostatically controlled room with cool white in fluorescent lamps at 2-3weeks.After incubation algal growth was measured by using UV-VIS spectrophotometer at 680nm [4].10ml from cultures were filtered under vacuum using filter membrane(0.45ųm) and washed several times with distilled water. Then, the algae cells were dried at 80°C for 30min and weighed.The fresh and filtered *C.vulgaris* samples were homogenized,

filtered and filtrate was evaporated under vacuum to dryness. The phenol content was estimated by the Folin-Ciocalteu procedure [5].

1g of sample was homogenized in acetone and allowed to stand over night at 4°C for complete extraction followed by centrifugation. The contents of total chlorophyll, chlorophyll a and b in the supernatant were spectrophotometrically determined [6].The total carotenoids [6] and phycocyanin [8] were spectrophotometrically determined at 450nm. It determined based on the [9] reduction of ferric to ferrous ions by tocopherol which then forms a red colour. Protein content in algal buffer extracts was assayed using comassein blue G 250 as a protein binding dye [10] Bovine serum albumin was used as a protein standard concentration in the sample was calculated from the calibration curve. It extracted from the cells with 2% mataphosphoric acid using di-chlorophenol indophenol dye [11].

Bacterial strains used for the antibacterial evaluation are *Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus* and *Klebsiella pnemoniae*. The antibacterial activity was evaluated with the paper disc diffusion method. The minimum inhibitory concentration tests were carried out according the method. MIC was defined as the lowest algal extracts concentrations showing no visible bacterial growth after incubation for 24h at 37°C.

RESULTS AND DISCUSSION

Table 1 shows the value of maximum specific growth rate (μ max) at 14days for *C.vulgaris* grown different $Nacl_2$ concentration. The results revealed that the concentration of $Nacl_2$ from1.50 to 2.15g/L^{-1} had significant influences on μ max. Lower $Nacl_2$ level (0.25 g/L) did not show any significant affect on the growth parameters. Similar to that was obtained [12], who demonstrated $NaNo_3$ concentration could be reduced to 0.625 g/L^{-1} without loss of productivity and leads to decrease production cost in large scale cultivation.

Table 1: Growth parameters and extraction yield of C.vulgaris grown at various $Nacl_2$ concentration

$Nacl_2$ (g L^{-1})	μ max (day $^{-1}$)	extraction yield %
2.15	0.85 ± 0.005	10.0
1.80	0.74 ± 0.004	14.3
1.50	0.63 ± 0.004	14.9
0.25	0.69 ± 0.003	15.2

The data refer to mean value ± standard deviation

Table 2 shows the influence of concentration on the growth and concentration of total carotenoids, chlorophyll, D-tocopherol, total phenol compounds, proteins and total phycocyanin. The highest concentration of carotenoid content was obtained in culture grown at lowest (0.25 g/L^{-1}) with value of 15.20 mg^{-1}. In contrast, the total chlorophyll and D-tocopherol content was found in culture grown highest at (1.80 gL^{-1}) with value of 14.35 and 1.32 mg^{-1}. [13] Reported that beta carotene and astaxanthine in cyanobacterium exposed to UV-B radiation exert their protective function as antioxidant to inactive UV-B induced radicals in photosynthetic membrane. On other hand, the carotenoids in the cell membrane of microalgae could act as a filter for UV-B radiation.

Table 2:Influence of varied $Nacl_2$ conc. on the levels of biochemical compounds

$Nacl_2$	Chlorophyll	Carotenoids	Phenol	Tocopherol	Protein	Phycocyanin	Vit-C Conc.
2.15	12.23±24	7.32±0.46	3.98±00	0.26±0.04	8.25±.00	30±1.14	3.55±70
1.80	14.35±1.2	8.25±0.35	4.23±0.21	1.32±0.05	3.90±.70	40±1.40	3.00±10
1.50	12.63±1.1	12.3±0.94	6.55±0.27	0.50±0.03	4.50±.00	45±1.23	2.10±40
0.25	8.12±0.9	15.2±0.98	3.22±0.25	0.40 ±0.00	5.85±.99	55±1.13	1.00±50

Values are significant at P=0.01

Both vitamin-C and total protein maximum content were obtained at 2.15 GL^{-1} with values of 3.98 and 8.25 mg^{-1}.Thus the highest concentration of phycocyanin with value of 55.70 and the highest phenol content at 6.55 gL^{-1}.This findings might explain with that the $Nacl_2$ is grown cells under higher $Nacl_2$ level required for synthesis of the amino acids, which makeup proteins and other cellular components such as chlorophylls and phycocyanin. However,

higher concentration of Nacl2 led to an increase level of biochemical compounds. These results were in agreement with those reported by several authors[14].

Antibacterial activity

The antibacterial of *C.vulgaris* extracts were assayed against for bacterial strains *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* and *Klebsiella pnemoniae* by evaluating the inhibition zones, zone diameter and MIC values. Generally, all *C.vulgaris* extracts were found to be effective against all tested bacteria and the antibacterial activities was found to be dose dependent .This phenomenon was in agreement with that found by the author [15].The data in table 3 showed that the most susceptible bacteria were *Klebsiella pnemoniae* and *Staphylococcus aureus to the organic extra*cts with highest inhibition zone ranged 4-9 mg/disc. It is of interest to note that all *C.vulgaris* organic extracts manifested similar degrees of susceptibility towards both gram positive and negative bacteria.

Table 3:Antibacterial activities- inhibition zone in diameter around the disc, MIC

Bacterial Strain	Disc (µg) grown in $2.15g^{L-1}$	Ab	MIC	Disc (µg) grown in $1.80g^{L-1}$	Ab	MIC	Disc (µg) grown in $1.50g^{L-1}$	Ab	MIC	Disc (µg) grown in $0.25g^{L-1}$	Ab	MIC
S.aureus	15	21	20	7	8	40	9	12	30	5	10	25
B.subtilis	16	22	20	4	7	40	8	10	30	4	09	25
M.luteus	14	19	20	4	6	40	8	11	30	4	07	25
K.pnemoniae	18	23	20	5	6	40	8	11	30	4	07	25

On the other hand, *C.vulgaris* extracts showed good potential of antibacterial activities against all of four bacteria with MICs ranged from 30-40µg/mL.The maximal inhibition zones and MIC values for bacterial strains sensitive to chloramphenicol as a standard antibiotic were in ranged of 13-20mm and 20µg mL^{-1} and with those published [16] mentioned that the antibacterial effect of biochemical compounds are in concentration dependent.

CONCLUSION

The present data indicates that the organic-extracts of *C.vulgaris* grown at different concentration possessed strongest antibacterial properties and may be considered as promising alternative source synthetic substances. In addition, their activities can be improved by changing the culture conditions.

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