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Effects of crude aqueous extract of *Bouhinia variegata* upon growth parameteres of *Chlorella vulgaris* Beijer

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Introduction

The new trends of research aims to use the plant natural products as a source of many useful compounds, which can be used in many applications to make the environments more safe and healthy. Some of these natural compounds are plant secondary products and plant extracts. In this study, the crude aqueous extarct (CAE) of *Bouhinia variegata* which characterized by presence of many chemical natural products as Myo-Inositol, 4-C-methyl ($C_7H_{14}O_6$), Octadecanedioic acid ($C_{18}H_{34}O_4$), Cyclopropanecarbonitril ($C_{18}H_{17}N_2Cl$) and 2,6-Diphenyl-4, 4-dihydropyridine-3, 5-dicarbonitril ($C_{21}H1_7\ N_3$) were used for treatments of *Chlorella vulgaris* Beijer cultures for 7 days. The CAE of *B. variegata* causes gradully stimulation of cell numbers and total pigments of *C. vulgaris* Beijer cultures when treated with 2 & 4 ppm of CAE of *B. variegata.* On the other hand, the dry weight, oxygen evalution and oxygen uptake increased up to the concentration of 4 ppm of CAE of *B. variegata.* However, under higher concentrations of CAE of *B. variegata*, the growth criteria (cell numbers, dry weight and total pigments), photosynthesis and some related metabolic activities are inhibited. The CAE of *B. variegata* is considered as a mixture of natural organic compounds can be used in the developments of the biological activities of *Chlorella vulgaris* Beijer cultures.

Materials and Methods

Tested alga: *Chlorella vulgaris* Beijer was collected from the Nile River and used as a tested organism. Beijerinck's nutritive culture was used as medium for enrichment and growth of the tested alga (Stein, 1966; Ahmed *et al.*, 1985; 1989).
Treatments: *Chlorella vulgaris* Beijer cultures were subjected to various concentrations mixtures (2, 4, 6, 8 and 10 ppm) of ACE of *B. variegata* for 7 days.

Analytical Method:
1-Phytochemical screening of CAE of *B. variegata*: Using GC/MS [Finnigan MAT SSQ 7000 mass spectrometer coupled with varian 3400 gas chromatography] according to Sharaf, *et al*. (2000).
2-Determination of cell numbers: Using Haemocytometer, (0.1 mm depth) having improved Naubuer ruling was used. One drop of the algal suspension was pipette on the covered side and left two minutes for alga setting. The mean counts of three replicates were taken into consideration and the results measured as cells ml^{-1}algal suspension.

3-Determination of dry weight: A definite volume (50-ml) of alga suspension was filtered through weighed glass fiber filter. The cells after being precipitated on filter were washed twice with distilled water and dried over night in the oven at 105 ^{o}C. The data were measured as μg ml^{-1} algal suspension.

4-Determination of photosynthetic pigments: The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using spectrocolorimeter. The contents of the pigment fractions (μg ml^{-1} algal suspension) were calculated using the equation mentioned by Metzner *et al.*, 1965.

5-Oxygen evolution: Using the site module 97.08 dissolved oxygen electrode as a polargraphic device, which described by Clark oxygen electrode (Lessler *et al*., 1956). Electrode separated from the magnetic stirred assay medium by a Teflon membrane (Oxygen membrane/electrolyte). The data of the oxygen evolution in the present study were calculated as μ mole O_2 ml^{-1}algal suspension hr^{-1}.

6-Oxygen uptake: The dark respiration was determined using oxygen uptake in the dark as indicator. At the end of oxygen evolution measurements, all the lights were switched off and the flasks were wrapped tightly in aluminum foil for complete darkness. Using the site module 97.08 dissolved oxygen electrode as a polargrahpic device described by Clark oxygen electrode (Lessler *et al.*, 1956). The results of oxygen uptake were calculated as μmole O_2 ml^{-1} algal suspension hr^{-1}.

RESULTS:

The data concerning the effect of crude aqueous extract of *B. varigata* upon growth criteria (cell numbers, dry weight and photosynthetic pigments), oxygen evolution and oxygen uptake of *Chlorella vulgaris* cultures, after being subjected to various treatments of (2, 4, 6, 8 and 10 ppm) of CAE of *B. variegata* were recorded.

The results of the cell numbers and total pigments of variously treated cultures are given in Table (1). Both the cell number and total pigments were increased gradually with lower and moderate concentrations (2,4 & 6 ppm) of CAE of *B. variegata*.

Table (1): Effect of crude aqueous extract of *B.varigata* upon cell number (cell ml^{-1} alga suspension) , dry weight (μg ml^{-1} algal suspension) and total pigments (μg ml^{-1} algal suspension) of *Chlorella vulgaris* Beijer for 7 days.

Treatments 00:ACE (ppm).	Cell number	% absolute control	Dry weight	% absolute control	Chloro. a	Chloro. B	Carot.	Total pigments	% absolute control
00:00	500 x 10^4	100.00	324.00	100.00	2.78	1.05	1.35	5.18	100.00
00:2	650 x 10^4	130.00	335.00	103.396	1.966	1.546	1.904	5.416	104.183
00:4	750 x 10^4	150.00	497.100	153.425	2.757	1.934	3.501	8.192	157.872
00:6	550 x 10^4	110.00	297.300	86.202	2.637	2.314	1.981	6.932	133.590
00:8	300 x 10^4	75.00	240.800	74.320	2.563	1.166	1.380	5.109	98.458
00:10	265 x 10^4	66.25	236.00	72.939	2.052	1.133	1.385	4.543	87.550

The maximum value of cell numbers is 150 % when treated with the concentration of (4 ppm) of CAE of *B. variegata*. The minimum value of cell numbers was considerably lowered to be 66 % when treated with the higher concentration (10 ppm)

of CAE of *B. variegata* comparing to the absolute control. The maximium value of dry weight is 153% under treatments with 4 ppm of CAE of *B. variegata*. The minimum value is 72 % under the level of 10 ppm of CAE of *B. variegata*. The maximum value of total pigments is 157% under treatments with 4 ppm of CAE of *B. variegata*, while the minimum value is 87% under the level of 10 ppm of CAE of *B. variegata*. These results were obtained comparing to the absolute control.

The results in Table (2) revealed that, oxygen evolution was promoted in cultures when treated to the lower concentrations (2 and 4 ppm) of CAE *B. variegata*. Under moderate and higher concentrates of (6, 8 and 10 ppm) of CAE of *B. variegata*, the oxygen evolution rate was inhibited. The maximum value of oxygen evolution is 200 % when treated with 4 ppm of CAE of *B. variegata***.** However, under higher concentrations (8 and 10 ppm) of CAE of *B. variegata*, the photosynthetic oxygen evolution was inhibited. The minimum value of photosynthetic oxygen evolution is 38 %. The results were obtained comparing to the absolute control.

The data at (Table 2) clearly demonstrate that oxygen uptake of *Chlorella vulgaris* was markedly promoted up to the level 4 ppm of CAE of *B. variegata*. The maximum

Table (2) Effect of crude aqueous extract of *B.varigata* upon oxygen evolution and oxygen uptake of *Chlorella vulgaris* Beijer for 7 dayes

Treatments 00 : ACE ppm	Oxygen evolution μ mole ml^{-1} algal suspension hr^{-1}	% absolute control	Oxygen uptake μ mole ml^{-1} algal suspension hr^{-1}	% absolute control
00:00	5.55	100.00	2.83	100.00
00:2	7.06	127.00	3.58	126.36
00:4	11.11	200.19	4.14	146.34
00:6	4.86	87.77	2.11	74.58
00:8	3.66	66.04	1.66	58.80
00: 10	2.13	38.47	0.99	35.05

value of oxygen uptake (146 %) was obtained with alga culture at 4 ppm of CAE of *B. variegata*. At relatively higher concentrations these contents were inhibited. The minimum value of O_2 uptake was extremely lowered (35 %) when treated with the level of 10 ppm of CAE of *B. variegata*. These previous results were obtained compared to absolute control.

DISCUSSION

The results of this study showed the effect of CAE of *B. variagata* upon growth parameters (cell numbers, dry weight and total pigments), photosynthesis and respiration of *Chlorella vulgaris* Beijere for 7 days. In vitro, many trials have been made to help the algae to overcome the pollution agents using variable treatments of some nature products from plants aiming to apply that results in further in the cultural fields.

The cell numbers and total pigments were gradually increased with various concentrations (2,4 and 6ppm) of CAE *B. variegata*. The maximum values of these contents were recorded under the level of 6 ppm of CAE of *B. variegata*. Under higher levels of CAE of *B. variegata* (8 and 10 ppm), the growth criteria (cell numbers, dry weight and total pigments), photosynthesis and respiration were inhibited

On the other hand, the dry weight, photosynthesis and respiration were increased up to the level of 4 ppm of CAE of *B. variagata*. The maximum values of these contents were recorded under the level of 4 ppm of CAE of *B. variagata.*

In conclusion, from the obtained results, we recommend treating some green algae with some plant natural products such as *B. variagata* crude aqueous extract in order to facilitate the reclamination of the desert lands polluted by heavy metals as well as purification of waste water. This kind of treatment leads to make our environments more safe and healthy.

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