Metabolomics and Biological activities of *Chlorella vulgaris* grown under modified growth medium (BG_{11}) composition

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ABSTRACT

The aim of this work was to determine the biochemical compounds and evaluate the biological activities of Chlorella vulgaris cultivated under a biotic stress condition (various Zinc and Cupper conc.). The growth rate was recorded as well as determination of active compounds, pigments and defense enzymes, in addition to the biological activities as antioxidant, antimicrobial and anticancer. The obtained results revealed that, higher copper concentrations [0.632 mg /L (Cu)] showed an inhibitory effect to growth while 1.76 mg /L (Zn) enhanced growth which reached its maximum at 25th day of cultivation. Furthermore, combination of 0.88mg/L (Zn) and 0.316mg /L (Cu) induced an increase in growth rate, catalase, tannins, lipid peroxidation and glutathione-S-transferase and a decrease in flavonoids, phenolic content, protein and antioxidant activity. Also, the results of antioxidant activity showed that, elevation of Zn conc. induced an augmentation of antioxidant activity either by DPPH(2, 2 diphenyl-1-picrylhydrazyl) or ABTS (2, 2'- azino-bis ethylbenzthiazoline-6-sulfonic acid), with maximum activity at 0.88 mg/L Zinc conc. (89.91%) even exceeded those of control (85.62%). While more elevated Zn conc. (1.76 mg/L) induced lower activity when compared with synthetic antioxidant standard (Butylated hydroxyl toluene, BHT). Concerning antimicrobial activity, Gram +ve bacteria, Staphylococcusaureus recorded moderate activity in sulfur-contained extract. Cytotoxicity of three cancer cell lines was inversely proportional to extracts conc. used, where the higher conc. (500µg/ml) showed the lowest cell viability of the tested cell lines which ranged from 22.06 to 69.89%. 0.316 mg/L (Cu) of conc. 500 µg/ml recorded the lowest

cell viability of (32.166 %) in breast cell line , (22.06%) in colon cell line, and 27.18 06%) in cervical cell line.

Keywords:*Chlorella vulgaris*, Chemical compositions, Anticancer, Antioxidant Antimicrobial, Defense enzymes

INTRODUCTION

Microalgae include unicellular, simple, primitive, and photosynthetic organisms and are considered a potentially valuable and new source of different biologically active molecules for applications in various fields such as food industry, agriculture, pharmaceutical, nutraceutical, and cosmetic sectors (Shalaby, 2011; Shanab et al., 2012; Shalaby and Dubey, 2018). *Chlorella sp.* is a microalga that potential by used for food supplement, pharmaceuticals, animal feed, aquaculture and cosmetics (Widayat et al., 2018). They can be easily cultured and harvested, have short generation times and enable an environmentally-friendly approach to drug discovery by overcoming problems associated with the over-utilization of marine resources and the use of destructive collection practices (Lauritano et al., 2016).

The bioactive compounds from natural sources has beneficial effects on health (Herrero et al., 2013) and used for the treatment of different human diseases (Newman and Cragg, 2012).

Several studies on the impact of various stresses including heavy metal exposure on microalgal growth have been done (Pinto et al., 2003; Zouari et al., 2016). Algae often minimize free radical damage by inducing an antioxidant defensive system (Li et al., 2006; Olivares et al., 2016), such as non-enzymatic (e.g., glutathione (GSH), tocopherols, ascorbate (ASC), and carotenoids), and enzymatic (e.g., superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX)), which are known to be involved in protecting plants against high toxic levels of heavy metals (Pinto et al., 2003; Sáeza et al., 2015; Machado and Soares, 2016; Moenne et al., 2016). A large number of algal extracts and / or extracellular products have proven antimicrobial, antitumor, antioxidant and antiviral activities (Ghasemi et al., 2004; Ozemir et al., 2004).

This study aimed to identify the secondary metabolites produced by *Chlorella vulgaris* cultivated undernutrient stress concentration especially copper and zinc as micronutrients by an increase or decrease of the element content in the culture medium (BG_{11}). Nutrient stress was performed by single nutritive component (Cu, Zn) and in combination of two elements (Cu+Zn) as well as the biological activities of these metabolites (antioxidant, antimicrobial and anticancer activities) were targeted in this study.