Produção e Controle de Produtos Naturais

Natiéli Piovesan
Vanessa Bordin Viera
(Organizadoras)

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NATIÉLI PIOVESAN
VANESSA BORDIN VIERA
(Organizadores)

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APRESENTAÇÃO

O Brasil possui uma das floras mais ricas e diversificadas do mundo – quase 19% da flora mundial. Nosso conhecimento sobre a diversidade, o cultivo e os benefícios que as plantas, frutos e sementes podem proporcionar ainda são incompletos. Dessa forma ressaltamos a importância de se continuar a explorar e conhecer o potencial que a flora brasileira possui.

Nesse intuito o e –book Produção e Controle de Produtos Naturais é composto por 13 artigos científicos que abordam assuntos de extrema importância relacionados à flora brasileira. O leitor irá encontrar assuntos que abordam temas como a atividade toxicológica de fungos, a composição química, biológica, atividade antioxidante, alelopática, citotóxica, anticitotóxica, teor de fenólicos totais e teor de flavonoides totais de plantas, além de fatores que podem ter influência sobre esses aspectos.

O e-book Produção e Controle de Produtos Naturais também apresenta artigos com intuito de orientação e incentivo ao uso, cultivo e manejo de plantas medicinais, além de temas relacionados à Gestão Ambiental e Sustentabilidade.

Diante da importância de discutir a biodiversidade, os artigos relacionados neste e-book, visam disseminar o conhecimento acerca da constituição da flora brasileira e promover reflexões sobre os temas. Por fim, desejamos a todos uma excelente leitura!

Natiéli Piovesan e Vanessa Bordin Viera

SUMÁRIO

CAPÍTULO 6

CHEMICAL PROFILE OF CRUDE EXTRACTS OF *ARTHROSPIRA PLATENSIS* BIOMASSES CULTIVATED IN DIFFERENT CULTURE MEDIA

Laura Patrício de Almeida Nunes Cavalcanti
LAPEMESP, Instituto de Pesquisas de Produtos Naturais, UFRJ, Av. Carlos Chagas Filho, 373, Prédio do CCS, Bloco H, Subsolo, Lab 18 SS, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ

Cláudia Maria Luz Lapa Teixeira
Laboratório de Biotecnologia de Microalgas, Instituto Nacional de Tecnologia, Av. Venezuela, 82 - Saúde, Rio de Janeiro, RJ

Roberto Carlos Campos Martins
LAPEMESP, Instituto de Pesquisas de Produtos Naturais, UFRJ, Av. Carlos Chagas Filho, 373, Prédio do CCS, Bloco H, Subsolo, Lab 18 SS, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ

ABSTRACT: *Arthrospira platensis* is a microalga which belongs to the group Cyanophyta; it has metabolites such as proteins, vitamins, carotenoids and the phycocyanin pigment, all of them showing a broad spectrum of biological activities. The Zarrouk medium, a chemically defined medium, is used for the growth and generation of compounds of interest in the algal biomass. However, a significative production cost is assigned to Zarrouk medium. This work aimed to conduct a comparative study between the Zarrouk culture medium and the modified F2 culture medium concerning to the production of products of industrial interest. TLC and HPLC-DAD techniques were used in order to verify the chemical profile of the biomass extracts (MeOH and EtOH) originated from the two different media and the production of their main bioactive compounds. TLC and HPLC results suggest that the extractions with EtOH led to a higher extraction of carotenoids and chlorophyll, as well as of other minor substances. In addition, biomass produced in Zarrouk medium seems to contain a greater amount of carotenoids than biomass produced in F2 medium.

KEYWORDS: *Arthrospira platensis,* HPLC-DAD, microalgae, chemical composition, culture media

1 I INTRODUCTION:

Arthrospira platensis is a blue green alga which is found in tropical and subtropical regions whose surface waters contain high levels of carbonates and bicarbonates in a pH range of 8 – 11. *A. platensis* belongs to the group Cyanophyta and is more specifically known as a cyanobacterium, which has a great pharmacological and alimentary value due to its rapid growth and a high production of metabolites, such as proteins, vitamins, carotenoids and the phycocyanin pigment, all of them showing a broad spectrum of biological activities (COLLA

*et al.,*1998; MACL *et al.*, 1999, MENDIOLA *et al.,* 2006; BOROWITZKA, 2013).

There have been a great number of relates of antifungal, antiviral, antialgal, enzymatic, or antibiotic properties of compounds extracted from *A. platensis*. Since their production changes according to the environment on which the microalga grows, manipulating the culture conditions can stimulate the biosynthesis of specific compounds (MORAIS *et al.,* 2015).

The indoor cultivation of this microalga has been a promising alternative, due to continuous production of microalga which does not follow harvest regimes. Besides, *in vitro* cultivation protect the growing cells of severe alterations due to the light and temperature outdoors. Additionally, standard culture medium might generate compounds of interest; medium can be reused, reducing water consumption and costs; the residual biomass derived from the extraction of the target compounds which can still be used as food supplements in feed or as fertilizer, among other advantages (LIMA *et al.,* 2018).

So far, microalgae *A. platensis* is cultivated in Zarrouk medium, a chemically defined medium containing minerals salts and nutrients necessary for the growth and generation of target compounds in the algal biomass. However, these chemicals are quite expensive, increasing the biomass production costs (RAJASEKARAN *et al.* 2015).

Modified media have been reported, using sea water and reuse water to optimize a specific target compound production, biomass yield or as an alternative as a low cost culture medium.

Leema *et al.* in 2010 reported the use of seawater as culture medium for *A. platensis* and Zarrouk medium was used as control. The seawater was pretreated with $NaHCO_3$ in order to precipitate the excess divalent cations Ca^{2+} and Mg^{2+}. Three different media were tested: SW1 (undiluted seawater), SW2 (2:1 seawater/freshwater, v/v) and SW3 (1:2 seawater/freshwater, v/v). The three seawater media supported the growth of *A. platensis*. In addition, SW2 showed significantly higher lutein content in the biomass than control medium.

Matos *et al.* in 2017 reported a desalination concentrate (wastewater) as an alternative method for microalgae cultivation. They showed that *A. platensis* is able to grow in desalination concentrate, which is rich in nutrients like N and P as well as other minerals (Cl^-, Ca^{2+}, Na^+), but had the production of protein lowered and lipids, amino acids and satured fatty acids increased. This fact is correlated to osmotic stress influenced by salt concentration.

Ho *et al.* in 2018 reported the optimization of the phycobiliprotein C-phycocyanin (C-PC), a protein with great commercial interest due to its wide spectrum of bioactivity activities, produced from *A. platensis*. In this paper, authors report light sources (white LED, monochromatic LED and fluorescent lamps) being manipulated to enhance C-PC productivity in *A. platensis* cultivated in Zarrouk recycled medium. The white LED resulted in higher C-PC production efficiency and the recycled medium proved to be an interesting alternative from an economic and environmental point of view for C-PC production.

Thus, the optimization and preparation of a low cost culture medium and the production of an extract largely enriched of commercially interesting biomolecules has been pursued by scientists over the years.

2 I OBJECTIVES:

Evaluate the chemical profile of ethanolic and methanolic extracts of *A. platensis* biomass cultivated in different media (Zarrouk and modified F2), showing qualitative differences in the production of carotenoids, chlorophyll, phenolic compounds and proteins.

3 I METHODOLOGY:

Microalgae *A. platensis,* initially provided by Professor Sergio Lourenço (Fluminense Federal University, Niteroi, RJ) from the Elizabeth Aidar Microalgae Collection, was cultivated in erlenmeyers containing liquid Zarrouk medium modified by George and F2 medium with the following modifications: superior concentration of nitrate and phosphate (equivalent to Zarrouk medium), and treatment with EDTA (for the removal of turbidity from precipitate of calcium salts) (GUILLARD, 1975; GEORGE, 1976). Both cultures were grown under fluorescent light 24h, T=22ºC, under constant stirring. At the final of the cultivation, biomass was recovered by filtration and lyophilized. Dried biomasses were extracted for 24h with MeOH:H_2O 8:2 (Z-MeOH and F2-MeOH) or 100% EtOH (Z-EtOH and F2-EtOH), separately.

The crude extracts obtained were analyzed by TLC (elution in ethyl acetate: Hexane 6: 4 and BAW 8:1:1) and HPLC-DAD (performed at the Analytical Center - IPPN, UFRJ. The analysis was performed using the following parameters: Phenomenex Column RP18 250 x 4,6 mm, 5mm; the mobile phase was a mixture of solvent A (water/ formic acid 0,1%) and solvent B (MeOH), at a flow rate of 0,9 mL min^{-1}; gradient: 0 min (30% B), 30 min (100% B), 45 min (10% B), 46 min (30% B); Total acquisition time: 55 min).

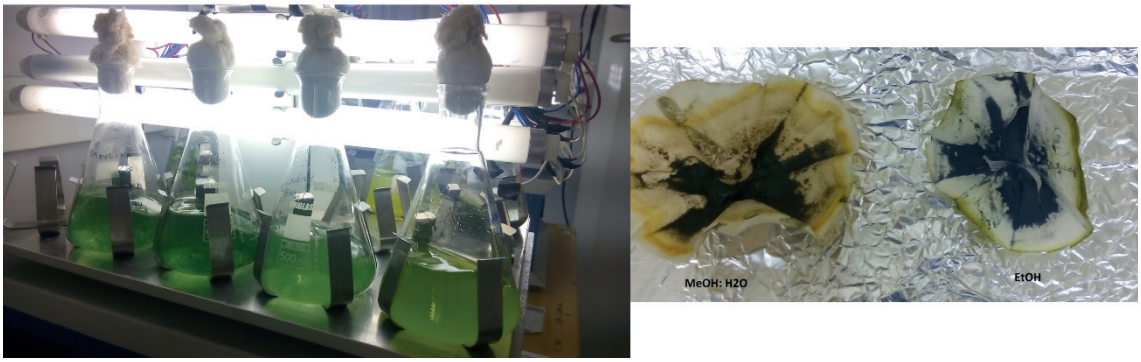

Figure 1: Cultivation of microalgae in erlenmeyer and biomasses of *Arthrospira platensis* (Zarrouk medium) after maceration.

4 I RESULTS:

Preliminary analysis by TLC, eluted in an apolar mobile phase confirmed the presence of β-carotene as the substance of higher Rf, confirmed by comparison with a standard sample of this carotenoid (LAGUNA *et al.* 2015). Phycocyanin and phenolic compounds were retained at the beginning of elution starter point due to their high polarity. In UV light, 360 nm range, it was possible to observe a very strong blue color only in the spot where the sample F2-MeOH was applied, which may suggest that this culture medium may have optimized the production of a more polar compound in the microalgae. In the case of the polar eluent, phenolics elute through the plate. As previously reported, at 360 nm, it was also possible to observe a very strong blue color in the spot where the sample F2-MeOH was applied (**Figure 2)**.

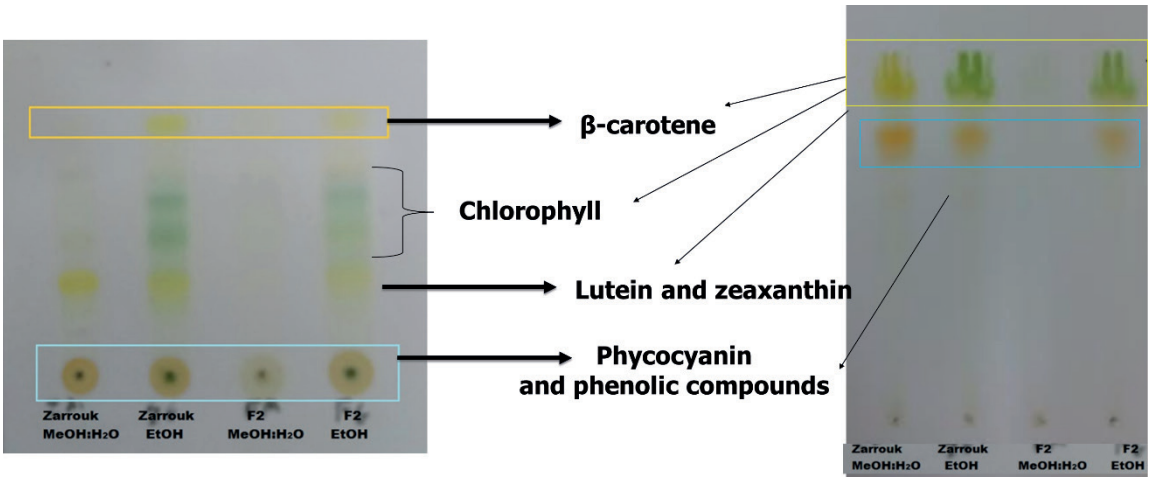

Figure 2: TLC of the crude extracts of *A. platensis* grown in Zarrouk and F2 medium (elution in ethyl acetate: Hexane 6: 4 and BAW 8:1:1).

Analysis by HPLC-DAD showed a massive concentration of substances in the ethanolic crude extracts. The crude extracts originated by Zarrouk medium showed a higher production of carotenoids, as confirmed by their UV spectra in 465 nm. TLC and HPLC experiments data suggest that the extractions with ethanol led to a higher extraction of carotenoids and chlorophyll, as well as of other minor substances.

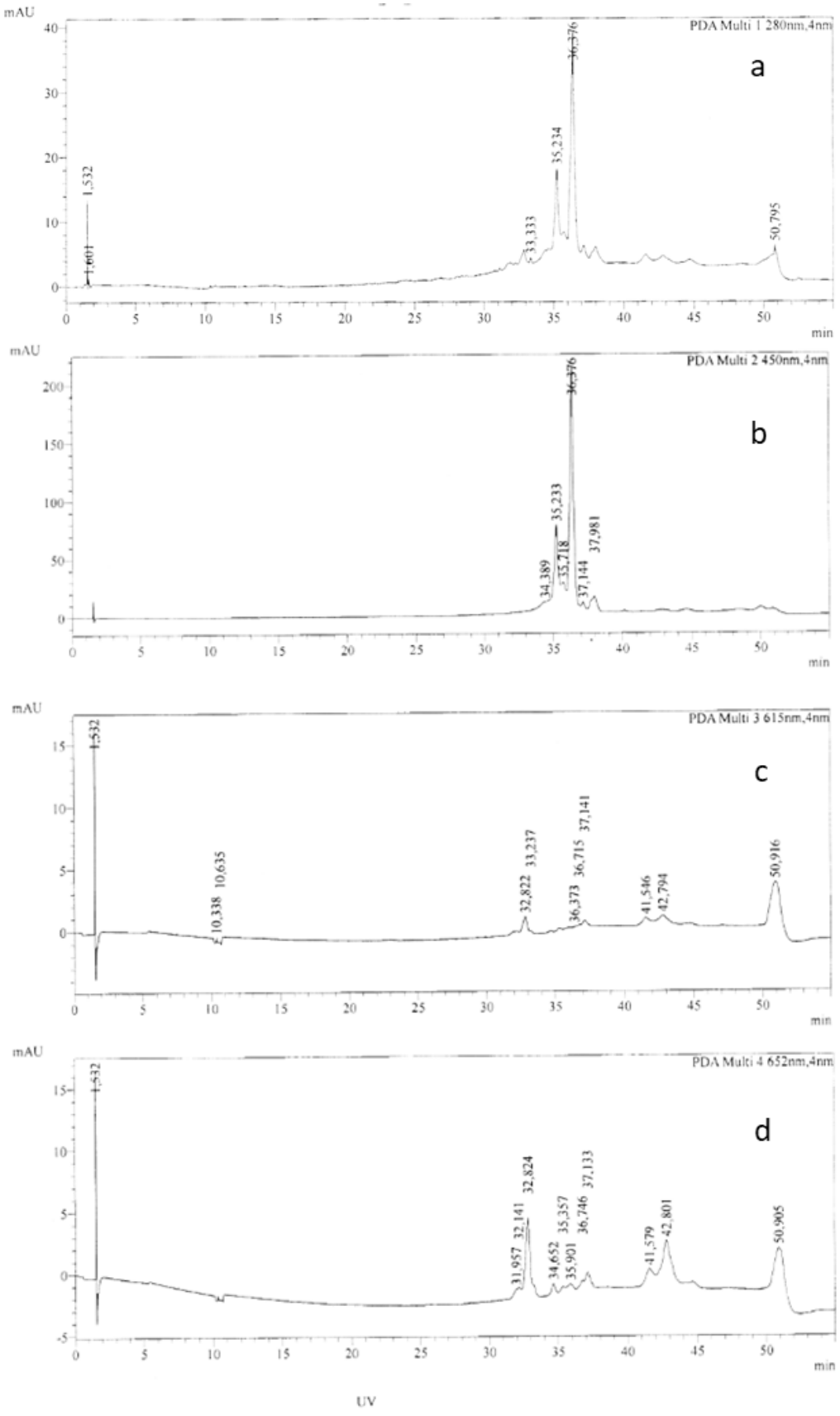

Figure 3: Chromatogram of the MeOH extract of *A. platensis* in ZK medium by HPLC-DAD, a) 280 nm; b) 450 nm; c) 615 nm; d) 652 nm.

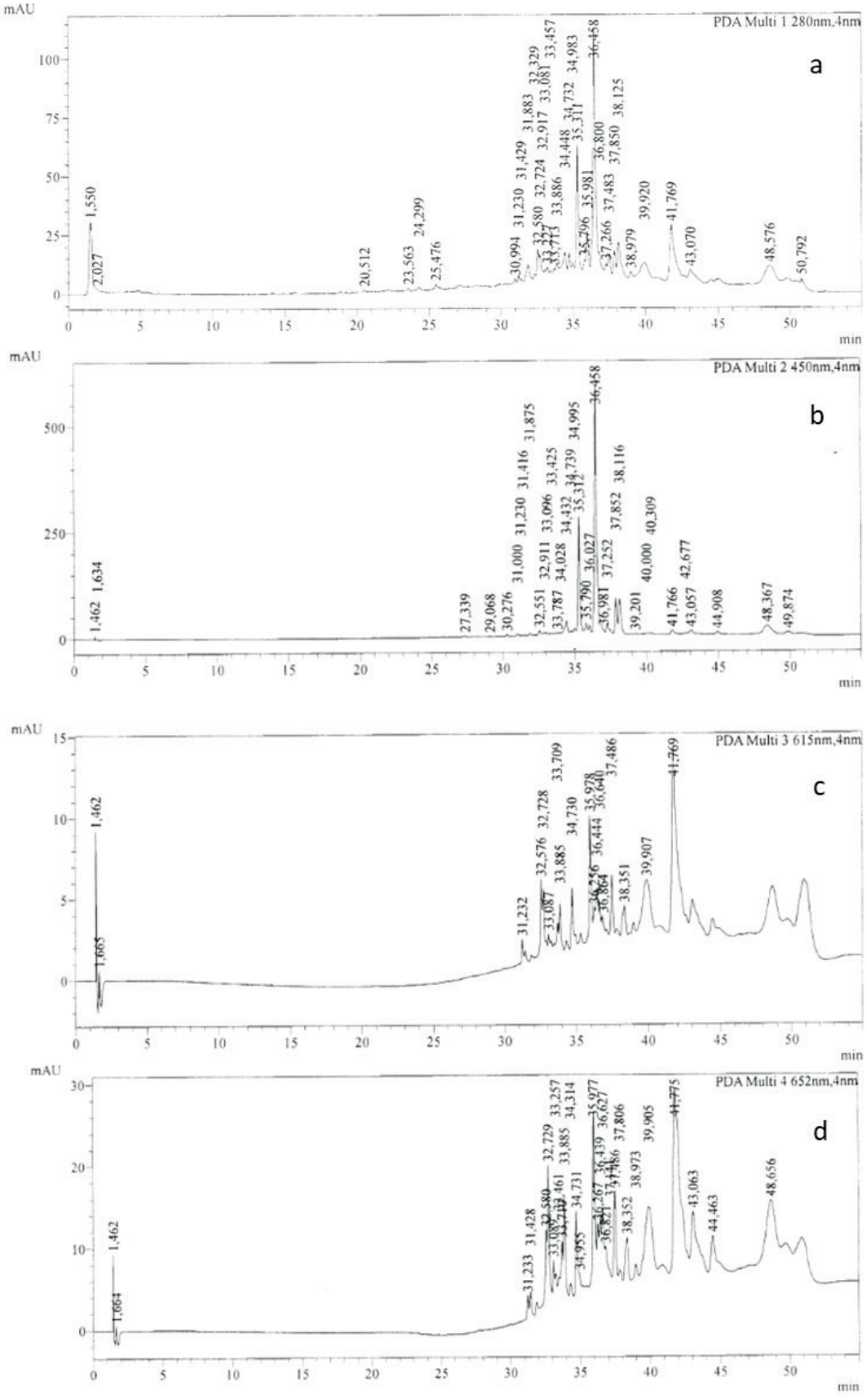

Figure 4: Chromatogram of the EtOH extract of *A. platensis* in ZK medium by HPLC-DAD, a) 280 nm; b) 450 nm; c) 615 nm; d) 652 nm.

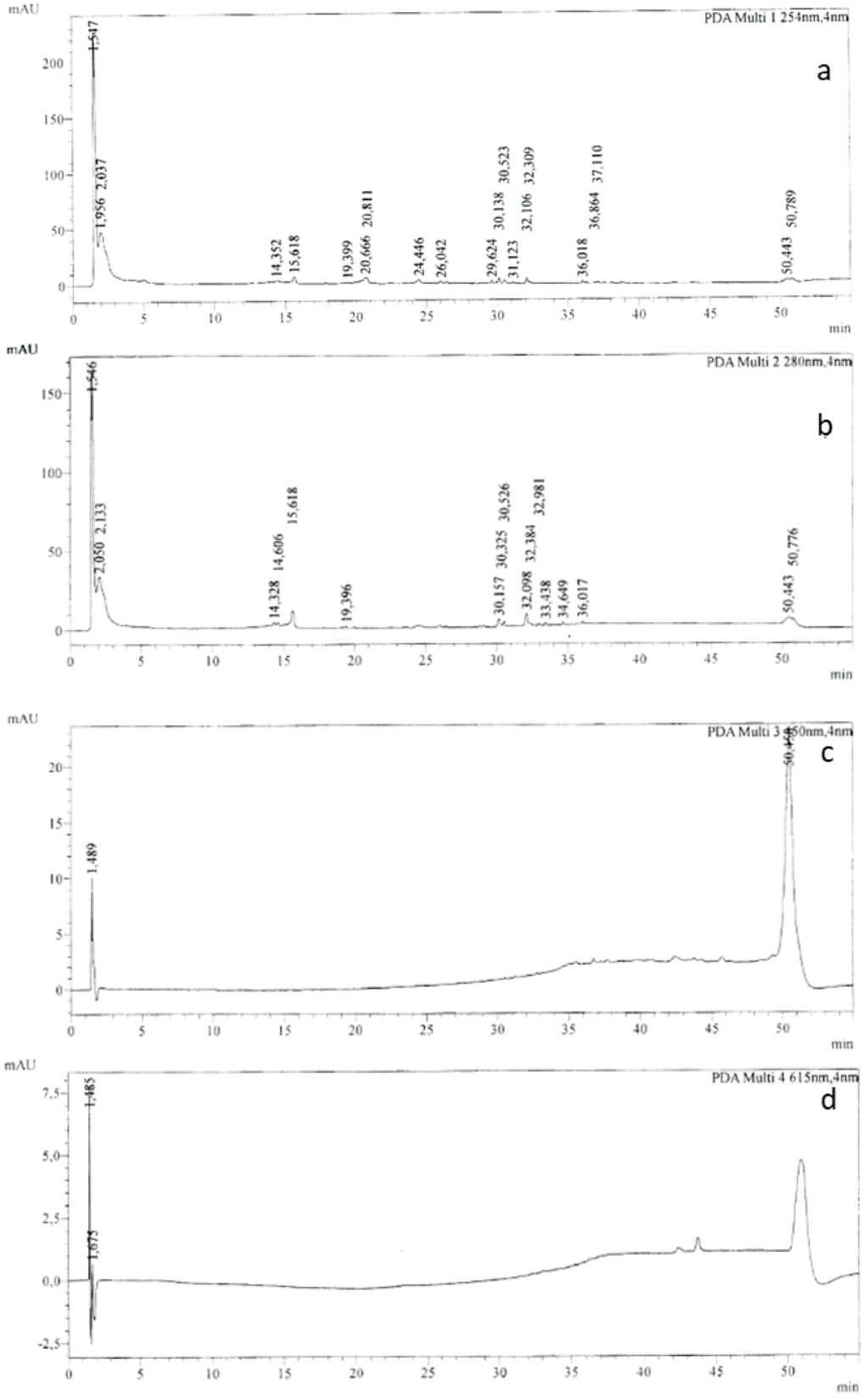

Figure 5: Chromatogram of the MeOH extract of *A. platensis* in F2 medium by HPLC- DAD, a) 252 nm; b) 280 nm; c) 450 nm; d) 615 nm.

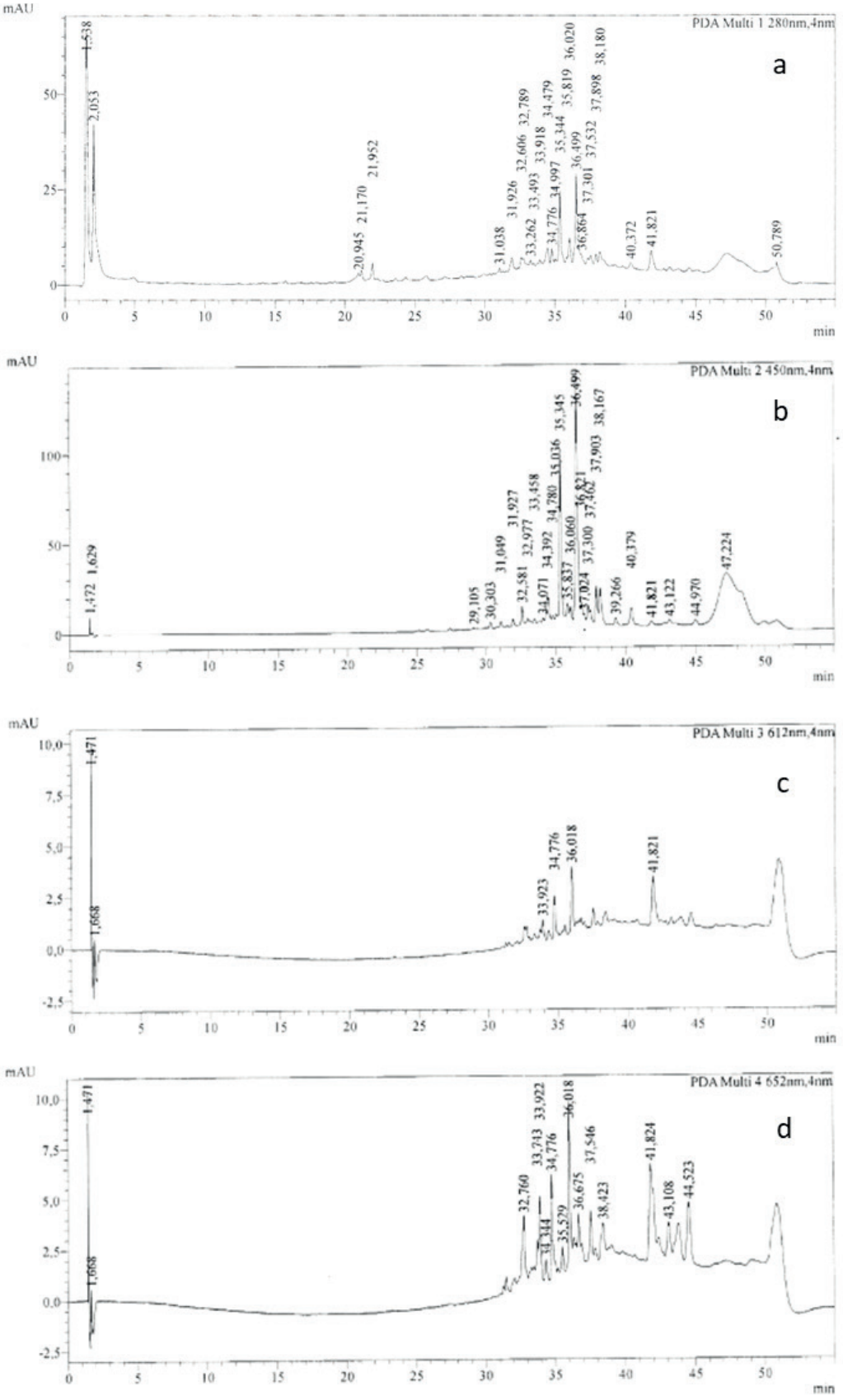

Figure 6: Chromatogram of the EtOH extract of *A. platensis* in F2 medium by HPLC- DAD, a) 280 nm; b) 450 nm; c) 615 nm; d) 652 nm.

5 I CONCLUSION:

Previous methodologies describe only the use of MeOH to obtain crude extracts of *A. platensis* biomass. Based upon our results, it is possible to suggest that extraction

with EtOH, a slightly less polar solvent seems to be an interesting alternative, since it can extract more molecules present in the biomass, mainly carotenoids. Zarrouk medium seems to produce a greater amount of carotenoids than F2 medium.

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