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Asian Journal of Plant Science and Research, 2012, 2 (4):546-549

ISSN : 2249-7412
CODEN (USA): AJPSKY

A comparative study of carotenoid extraction from algae in different solvent systems

Chitta Ranjan Sarkar*, Lima Das, Bagmita Bhagawati and Bhabesh Ch. Goswami

Department of Chemistry, Gauhati University, Guwahati-781014, Assam, India

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ABSTACT

Algae are a rich source of carotenoids. Carotenoids from algae can be used as a food colorant, food additive, cosmetics, antioxidants and nutraceuticals etc. Selection of solvents for extraction of carotenoid from algae is a tedious job and is generally carried out in organic solvent. In this study, different solvents ranging from organic to aqueous and their mixture were used to achieve the maximum extractability of total carotenoids. The extracted total carotenoids were estimated using UV- visible spectrophotometer and identified by Reversed Phase High Performance Liquid Chromatography (RPHPLC).

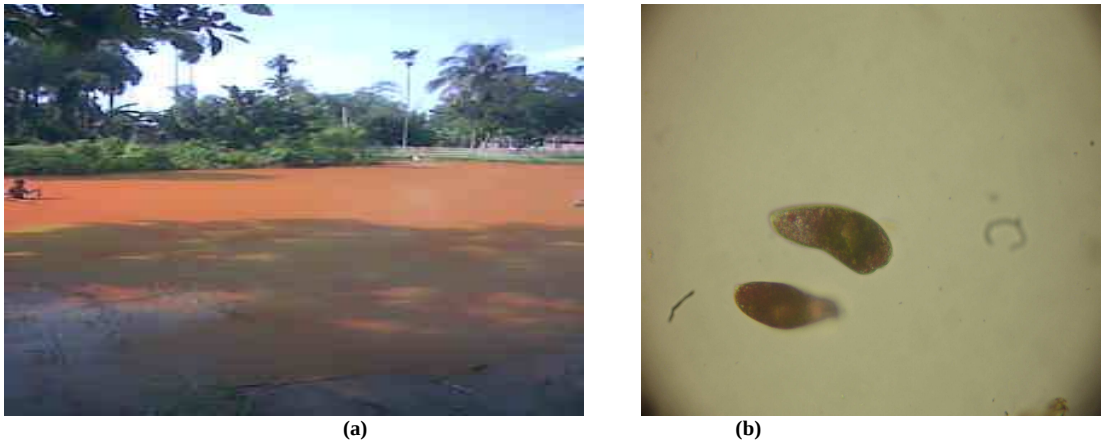
Key words: Algae, carotenoid, antioxidant, UV-visible spectrophotometer, HPLC

INTRODUCTION

Algae are a rich source of carotenoids [1] which are natural pigments, comprising a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). Carotenoids are excellent singlet oxygen scavenger and are used as food colorant, food additive, cosmetics, nutraceuticals etc [2,3]. The micro algae have a world wide distribution and able to survive under different environmental stresses including heat, cold, photo oxidation, uv exposure etc [4]. Carotenoid extracts are desirable for use in dietary supplements and as additions to processed foods. Extraction of carotenoid is generally carried out with organic solvent. A good extraction procedure should release all the carotenoids from the sample and bring them into solution without causing any change in them. As the short chain alcohol especially ethanol and isopropanol, have been proposed as alternative extracting solvents due to their greater safety and reduced probability of regulation [5], so the use of these solvents with non aqueous organic solvents may be suitable for extraction of carotenoid.The selection of the solvent to promote the extraction is a very important issue since it determines the degree of affinity to the chemical composition of the substances to be extracted. In this work mechanical procedures like vortexing and homogenizing in different solvents and their mixtures were used to maximize the extraction.

MATERIALS AND METHODS

MATERIALS:
Algae available in local fishery ponds in Kamrup district of Assam, India were taken as source of carotenoid for extraction. Red Algal scum was collected from the fishery pond (Fig 1a), the algal mass was washed with water and then freeze dried for a period of 12 hours. A part of the algal sample was identified under microscope (LABOMED ATC-2000) and it was found to be *Euglena* (Fig1b)



(a) **(b)**

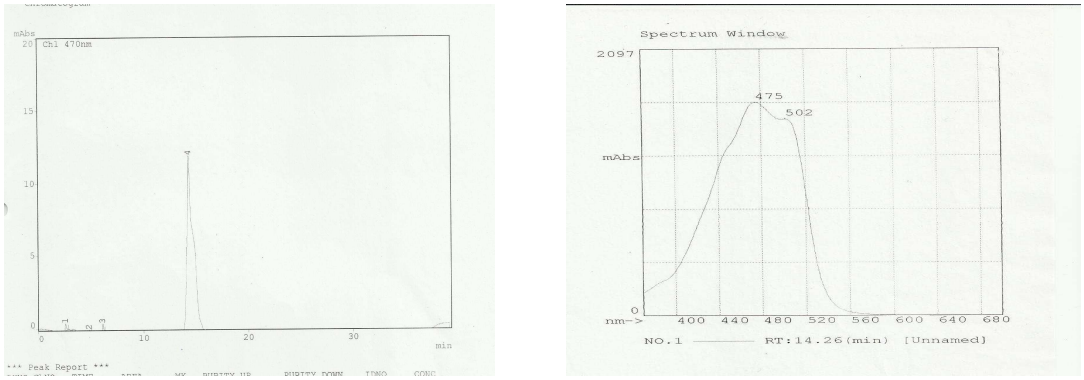
Fig 1. (a) Collection of algae (b) Microscopic picture of *Euglena*: 40X magnification.

In our experiment all the chemicals and solvents used for extraction were of analytical grade and solvents used for HPLC were of HPLC grade purchased from Qualigens Fine Chemicals, Mumbai, India. Lycopene standard was purchased from Sigma Aldrich, USA.

METHODS:

(a)Identification of major carotenoid present in the studied algae

The freeze dried algae was grinded with anhydrous Na_2SO_4 and extracted with acetone using BHT as antioxidant. The major carotenoid was purified by TLC. The purity was checked and identified by RPHPLC (SPD-M 10 AVP, SHIMADZU). The major carotenoid Lycopene was identified on the basis of retention time and UV absorption spectra of standard Lycopene. (Fig 2a, 2b) All the experiments were performed under dim light and in N_2 atmosphere.



(a) **(b)**

Fig2. (a) HPLC Chromatogram of purified Lycopene extracted from algae sample showing the Lycopene peak at 14.26 min and (b) its UV absorption spectra. Column; LC-8, 25 x 4.6 mm, 5 µm column, mobile phase CH_3CN: H_2O (97.5: 2.5) &CH_3CN: DCM (70: 30)

(a)Experimental design

Extractions of carotenoid were carried out in different procedures viz grinding followed by vortexing with glass beads, grinding followed by homogenization using different solvents and mixture of solvents. Each extraction was carried out with 0.052g dried algae. 20ml of each solvent in four equal fractions were used for extraction. The total carotenoid present in the extract was measured by UV-visible spectrophotometer (UV- 1601 SHIMADZU) and estimated using the following equation [6]

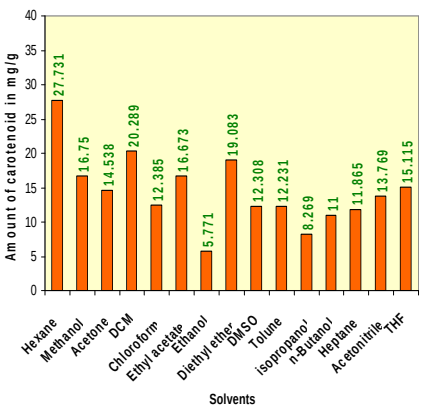
$$\text{Total carotenoid} = \frac{OD \times TV}{E^{1\%}_{cm} \times 100} \times 10^6 \ \mu g$$

OD = optical density, TV = total volume, $E^{1\%}_{cm}$ =extinction coefficient

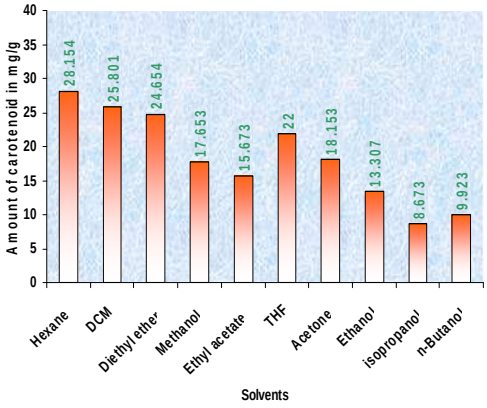
RESULTS AND DISCUSSION

Set 1

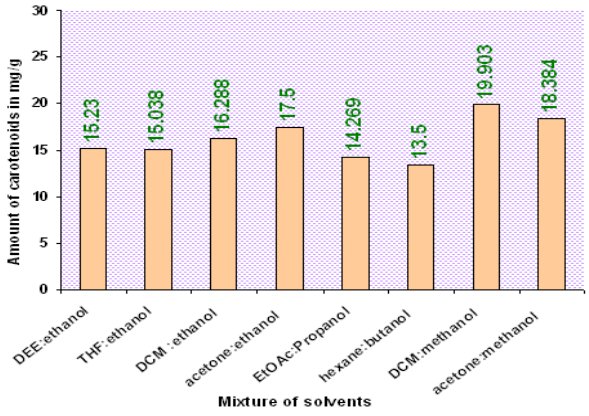
The weighed algae was grinded and then vortexed with glass beads using hexane, methanol, acetone, dichloromethane (DCM), chloroform, ethyl acetate, ethanol, diethyl ether, dimethyl sulfoxide (DMSO), tolune, isopropanol, n-butanol, heptane, acetonitrile and tetra hydro furan (THF).In this set of experiment the maximum amount of carotenoid was extracted in hexane and the least extraction was in ethanol (Fig 3a).In case of ethanol, isopropanol and n-butanol, the color of the residue were found to be red which indicates the incomplete extraction of carotenoid in these solvents.



(a)



(b)



(c)

Fig 3 Amount of carotenoid in (a) grinding-vortex (b) grinding-homogenization (c) mixtures of solvents

Set 2

In this set of experiment extractions ware carried out after grinding and homogenization of the algae samples with hexane, DCM, diethyl ether, methanol, ethyl acetate, THF, acetone, ethanol, isopropanol and butanol. Other solvents used in set 1 were not included in this set as their extractability is less and some of them are also irritating. Although the extraction of carotenoid with ethanol, isopropanol, and n-Butanol were poor, yet they were used in this set considering their aqueous nature. The amount of carotenoid in different solvents in set 2 is shown in (fig 3b). Extraction after homogenization is found to be better as compared to extraction after vortexing in almost all the solvents. This might be due to the breaking of cell wall of algae in homogenization.

Set 3

In this set of extraction we tried to extract the carotenoids in mixture of solvents containing at least one aqueous solvent. The various solvents used in mixture are DEE, ethanol, hexane, acetone, THF, DCM, ethyl acetate, isopropanol, n-butanol and methanol (Fig3c). In the mixtures 30% of aqueous solvents were used. While using

hexane-ethanol/methanol mixtures two layers were formed in the homogenized extract for which these mixtures were not used further. In case of hexane- n-butanol mixture single layer was formed in the homogenized extract. Since the amount of total carotenoids extracted with acetone-methanol/ethanol mixture were almost same with that of acetone, so it would be better to use these aqueous mixtures (which are green solvent) instead of acetone alone.

CONCLUSION

Most of the studies of carotenoid extraction is based on single solvent extraction. Ethyl lactate is a good solvent for extraction of both cis and trans isomer of Lycopene [7]. In this study we have considered some easily available solvents including both aqueous and non aqueous. We have also selected some physical methods like use of glass beads, homogenization and we got better results in both cases as compared to that of grinding only. However homogenization is better than use of glass beads. It may be due to break down of cell wall of alga. In case of homogenization hexane, DCM, DEE, THF showed better extractability. When we mixed up these solvents with ethanol, methanol, butanol, extractability is decreased. However, when acetone: methanol/ethanol (7:3) is used the extractability was found to remain same as that extracted with acetone only. As acetone methanol/ethanol are green solvents, so acetone: methanol/ethanol (7:3) solvent system may be used for better extraction of carotenoid. As the carotenoids are used as food additive so their extraction in solvent mixtures containing aqueous solvents (ethanol/methanol) would be preferable to organic solvent alone.

Acknowledgments
The authors would like to thank Department of Chemistry, Gauhati University,UGC NERO(Assam). Rajiv C.Dev Goswami Department of Biotechnology, Gauhati University.

REFERENCES

[1] I Jaswir, D Noviendri, R F Hasrini & F Octavianti, *J. Medicinal Plants Research*, **2011**, 5(33), 7119-7131.
[2] M R Fazeli, H Tofighi, N Samadi, H Jamalifar, A Fazeli, *DARU*, **2006**, 14(3), 146-150.
[3] P C Sharma, S Jain, G Yadav, A Sharma, *Der Pharmacia Sinica*, **2010**,1 (3),95-108.
[4] A C Guedes, H M Amaro, F X Malcata, *Mar. Drugs*, **2011**, 9, 625-644.
[5] N Othman, Z A Manan, S R W Alwi & M R Sarmidi, *J. Applied Science*, **2010**, 10(12), 1187-1191.
[6] R G A Oliveira, M J L Carvalho, R M Nutti, L V J Carvalho & W G Fukuda, *African J Food Science*, **2010**, 4(4), 148-155.
[7] B K Ishida, M H Chapman, *J Agric. Food Chem*, **2009**, 57(3), 1051-1059.