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Extraction, Anti-Oxidant, Anti-Bacterial and Anti-Cancer Activity of Lutein from Microalgae

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Abstract

The aim of the study is to extract lutein from microalgae (*Chlorella* spp. and *Scenedesmus* spp.) and study its anti-oxidant, anti-bacterial and anti-cancer activity. Lutein was extracted by isolating and cultivating *Chlorella* spp. and *Scenedesmus* spp. and by using *Chlorella* spp. powder. *Chlorella* spp. was cultivated in two types of media namely Chu's 10 media and Basal Bold media (BB) media. The cultivation was carried out for a period of two months. Alkaline treatment (using KOH) was done and then followed by 3-step di-ethyl ether extraction. Lutein was extracted using three different solvents namely acetone, methanol and butanol and without any solvent but using olive oil. Test for carotenoids was done in order to confirm the presence of carotenoids. Anti- bacterial test (well agar diffusion) was done in order to find whether lutein was active against the bacterial organism (*Staphylococcus* spp. and *E. coli.*). Anti-oxidant assay {Total Anti-oxidant Capacity (TAC) and H_2O_2 anti-oxidant assay} was done to find the anti-oxidant activity. Anti-cancerous activity (MTT assay) was done in order to study the anti-cancer properties of lutein extracted from micro-organism. Gas Chromatography- Mass spectroscopy (GC-MS) was done to find the concentration of lutein present in the algal samples. The extraction of lutein from microalgae will provide a new source of lutein derived from micro-organisms.

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Introduction

Lutein is a carotenoid vitamin which is synthesized only by plants and photoautotrophic organisms like microalgae. Lutein is found in egg yolks and animal fats. The human retina accumulates lutein. It has physiological role in improving vision and in eye protection from harmful UV light. *Chlorella* spp. and *Scenedesmus* spp. can serve as a potential source of lutein because its photosynthetic efficiency. The extraction of lutein from microalgae will provide a new source of lutein derived from micro-organisms.

Lutein production is currently from marigold flowers and there is no commercial lutein production uses microalgae. Lutein is used as an food colorant. Lutein causes the yellow color of chicken skin and fat, and is used in chicken feed for this purpose. Lutein was used in chicken feed to improve the color of broiler chicken skin. Lutein is used as an alternative medicine. Lutein reduces the risk of age-related macular degeneration (AMD) and cataracts.

Materials and methods

Sample collection, isolation and cultivation of *Chlorella* species and *Scenedesmus* species

Samples were taken from a lake in Vellore and they are identified to be *Chlorella* spp. by viewing the algal sample under a microscope. The microalgae *Chlorella* spp. and *Scenedesmus* spp. was cultured in Chu's 10 media and Basal Bold media.

Extraction of lutein

2 ml of algal mass was taken and to it 20 ml of KOH was added.2 ml of di-ethyl ether was added in each step of di-ethyl ether extraction. 0.5 ml of acetone was added and kept in a magnetic stirrer at 40ºC for 10 minutes. It was then re-dissolved in 0.5 ml of olive oil. Thus lutein oil is obtained.

Test for carotenoids

The color of the solution containing the solvent disappears after the successive addition of 5% solution of sodium nitrate and 0.5M H_2SO_4.

Anti-bacterial activity: Agar well- diffusion method

MHA was prepared (1.14g of MHA dissolved in 30ml of distilled water). *Staphylococcus* spp. and *E. coli* broth are taken. They swapped on the agar plate after the solidification of the agar.

Anti-oxidant activity

Total anti-oxidant capacity (TAC)

1ml of lutein was mixed with 3 ml of the TAC reagent and incubated for 10 minutes. The readings were taken using colorimeter at 695 nm.

TAC reagent composition (50ml)

- Ammonium molybdate- 0.25g.
- HCl- 1.5 ml.
- Sodium sulphate- 0.2g.

H_2O_2 anti-oxidant assay method

A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4).The concentration of H_2O_2 was determined by absorption at 230nm in a spectrophotometer. The extract was added to H_2O_2 solution (0.6 ml, 40mM).

Phosphate buffer composition (12 ml)

Sodium di hydrogen phosphate – 3.475g.
Di sodium hydrogen phosphate- 6.71g.
One drop of H_2O_2 was added.

The absorbance was read at 230nm after 10 minutes against the blank solution without H_2O_2.

Inhibition % = (A of control-A of test/A of Control) × 100.

Column chromatography

Silica gel was suspended in hexane to the slurry at a concentration of 5% (w/v). The amount 5 grams of silica gel slurry prepared was packed into a column and 0.5 ml of lutein was then loaded into the column separately. Hexane: ethyl acetate (70:30 v/v) was used as a mobile phase. Fractions collected were given for GCMS analysis.

Gas Chromatography- Mass spectroscopy (GCMS)

The concentration of lutein present in the extracted lutein from algae was found by GC-MS.

Anti-cancer activity

MTT assay

MCF-7 breast cancer cell line was used to study the anti-cancerous activity of lutein.

Materials needed: Dulbecco's Modified Eagle's Medium (DMEM), Breast cancer cell line- MCF-7, 96 well tissue culture plate, Dimethyl sulfoxide (DMSO), MTT (1M of PBS in 5mg/ml of MTT).

Procedure

Day 1-75µl of DMEM was added on to the 13 wells. Incubation was done for 24 hours.

Day 2- 50µl of old DMEM was replaced by new 50µl DMEM. Incubation was done for 42 hours.

Day 3- 50µl of the DMEM with the sample was taken

from the wells except the control. 50µl of MTT was added to the wells except the control. 50µl of DMSO was added to the wells except the control.

Results and discussion

Sample collection, isolation and cultivation of *Chlorella* species and *Scenedesmus* species

Samples collected were identified to be *Chlorella* species. The microalgae *Chlorella* species and *Scenedesmus* species were cultured in Chu's 10 media and Basal Bold media.

Extraction of lutein

Lutein extracted from *Chlorella* species powder gave the highest concentration of lutein.

Test for carotenoids

The color of the solution containing the solvent disappeared after the successive addition of 5% solution of sodium nitrate and 0.5M H_2SO_4.

Fig. 1: Test for carotenoids.

Anti-bacterial activity of standard lutein

Staphylococcus sp.

No zone of inhibition was seen indicating that standard lutein was not active against and *E.coli.*

Fig.2: *Staphylococcus sp.* **Fig.3:** *E. coli*

Anti-bacterial Activity of lutein extracted from cultivated *Chlorella* species

Staphylococcus species

No zone of inhibition was seen indicating that lutein extracted from cultivated *Chlorella* species was not active against *Staphylococcus* species and *E. coli.*

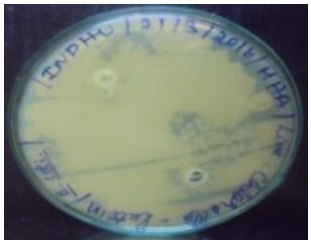

Fig.4: *Staphylococcus sp.* **Fig.5:** *E. coli*

No zone of inhibition was seen indicating that lutein extracted from cultivated *Chlorella* spp. was not active against *E. coli.*

Anti-oxidant activity

Total anti-oxidant capacity (TAC)

Samples	Control	Test
Standard lutein	-	0.69
Olive oil	0.65	0.69
Acetone	0.65	0.69
Methanol	0.67	0.71
Butanol	0.67	0.70

The anti-oxidant activity of lutein extracted using methanol as a solvent was found to higher when compared to other solvents.

H_2O_2 anti-oxidant assay method

Samples	Control	Test
Standard lutein.	1.270	1.272
Lutein extracted using olive oil from *Chlorella* species powder	1.271	1.273
Lutein extracted using acetone from *Chlorella* species powder	1.272	1.272
Lutein extracted from cultivated *Chlorella* species	1.273	1.278

The anti-oxidant activity was found to higher in lutein extracted from cultivated *Chlorella* spp. than lutein extracted using olive oil and acetone.

Column chromatography

Standard lutein, lutein extracted from *Chlorella* species powder using acetone and olive oil was purified.

Gas Chromatography- Mass spectroscopy (GCMS)

Lutein extracted from *Chlorella* spp. using olive oil (72.11%) was found to have higher concentration of lutein than lutein extracted from cultivated *Chlorella* spp. (65.69%).

GC-MS analysis of lutein

The peak area percentage and peak area coverage of the Lutein is given below.

Sample	RT (mins)	Peak area	Peak area (%)
Std. lutein	1.08	3971840	100
Lutein extracted from *Chlorella* sp. powder	1.12	2864256	72.11
Lutein extracted from cultivated *Chlorella* sp.	1.08	2609456	65.69

Lutein extracted from *Chlorella* spp. powder (72.11%) using olive oil has higher amount of lutein than lutein extracted from the cultivated *Chlorella* spp. (65.69%).

Anti-cancer activity

MTT assay

Colour change was observed in standard lutein, lutein extracted from *Chlorella* powder which indicates that they exhibit anti-cancerous property. No colour change was observed in the lutein extracted from cultivated *Chlorella* species which indicates that it does not exhibit anti-cancerous property.

Samples	Concentration (75µl)	Concentration (100µl)
Std. lutein	121.20	150.87
Acetone	301.74	436.41
Methanol	332.92	392.52
Cultivated *Chlorella* sp.	230.42	260.35

Lutein extracted by using acetone showed the highest inhibition percentage followed by methanol, cultivated *Chlorella* species and standard lutein.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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