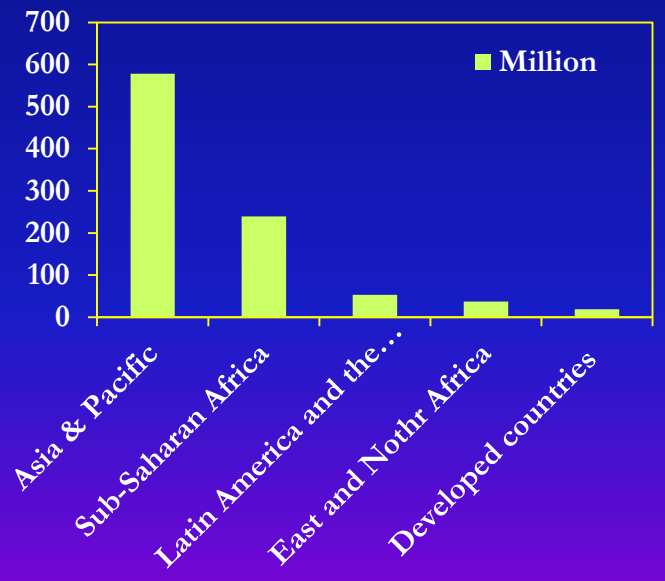
APPLICATION OF CAROTENOIDS WITH SPECIAL REFERENCE TO MICROALGAE

Dr. Ranga Rao Ambati
Assistant Research Professor
Department of Science and Technology
Beijing Normal University-Hong Kong Baptist University
Zhuhai, Guangdong, P.R.China
***e-mail:arangarao99@gmail.com**

TYAN First International conference
Rio de Janeiro, Brazil 22-24 August 2017

Global issues-Hunger and Malnutrition

- 60% of deaths occurred due to Hunger and Malnutrition
- 70% of hunger lives in rural areas
- 7.6 million children died from hunger
- 98% of world wide hunger exists in under developed countries
- 60% of hunger exists in Asia/South Pacific
- Vitamin A deficiency affects on 25% of the developing world's preschooler's-blindness, diseases, high mortality
- ***A 684,000 child deaths worldwide could be prevented by vitamin A and nutrients***



www.wfp.org

Why Microalgae?

- A 30k species of algae living every where
- Grown in marine or fresh water systems
- Cultured in autotrophic and heterotrophic culture conditions
- Accumulates carotenoids, lipids, fatty acids, proteins, amino acids, carbohydrates, vitamins and also minerals..etc
- Used in food, feed, nutraceutical and pharmaceutical applications

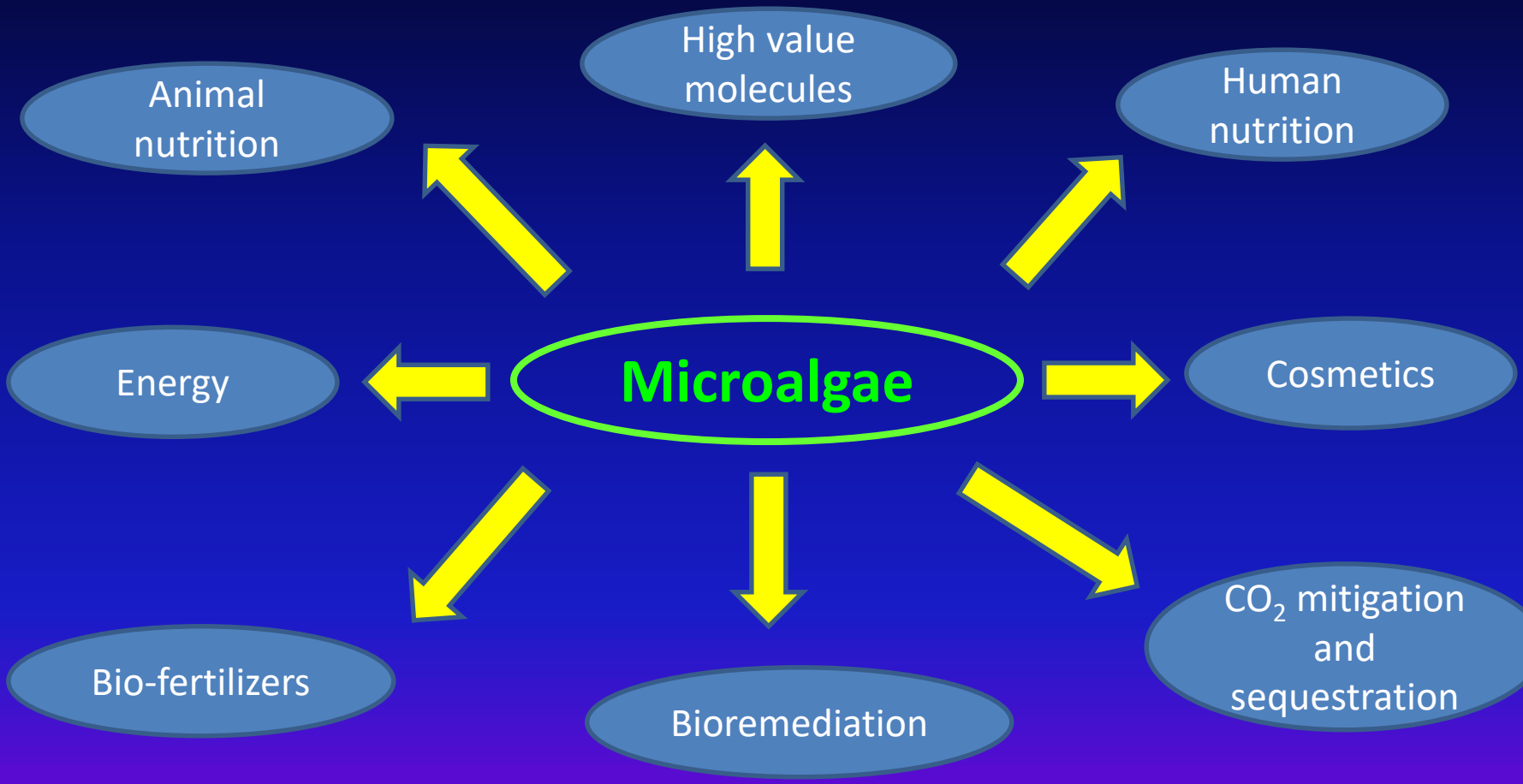
Mata et al., 2010; Wells et al., 2017

What are the potential algae species

- *Haemtococcus*
- *Spirulina*
- *Dunaliella*
- *Scenedesmus*
- *Chlorella*
- *Nannochloropsis*
- *Isochrysis*
- *Prophyridium*

Algal cultivation systems

Applications of algae



Algae products in the market

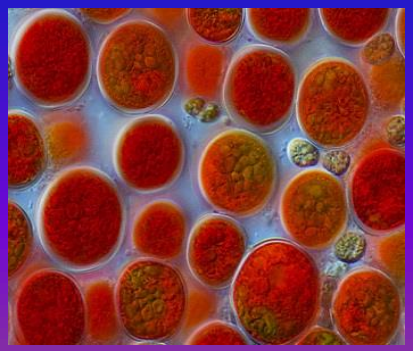
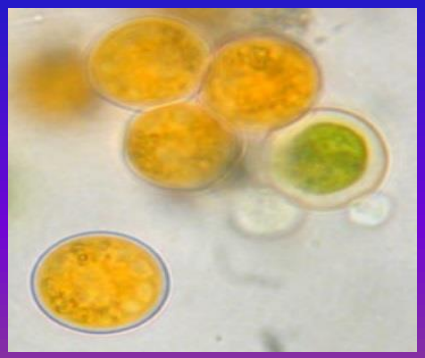
Carotenoids

- Natural pigments
- Two groups-Hydrocarbons and oxygenated xanthophyll's
- Biosynthesis by plants, algae, fruits, vegetables and birds..etc
- Plays major role in photosynthesis and photo protection
- Ability inactivate reactive oxygen species (ROS)
- Precursors of vitamin A
- Showed antioxidant properties
- Used in biological activities

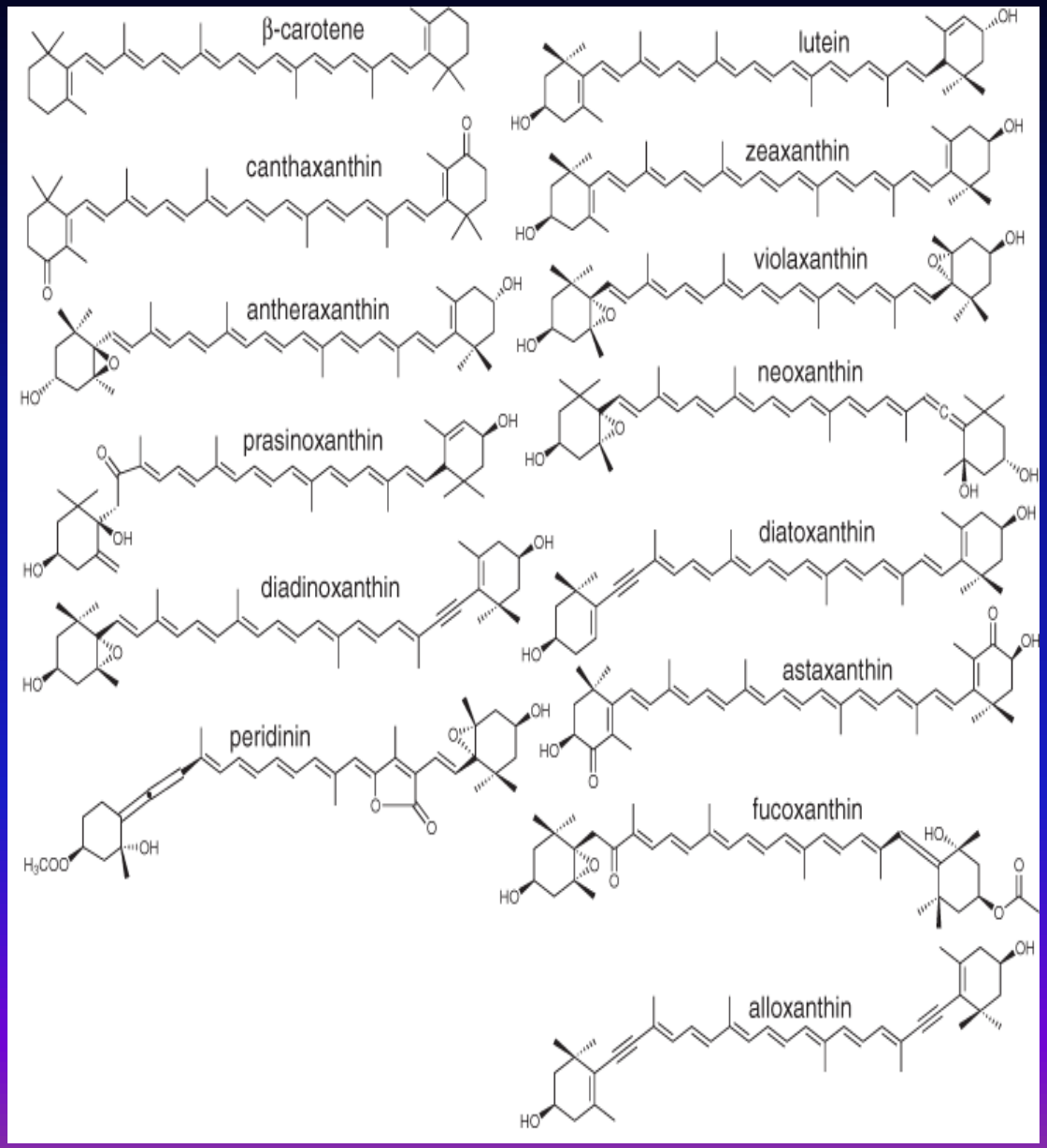






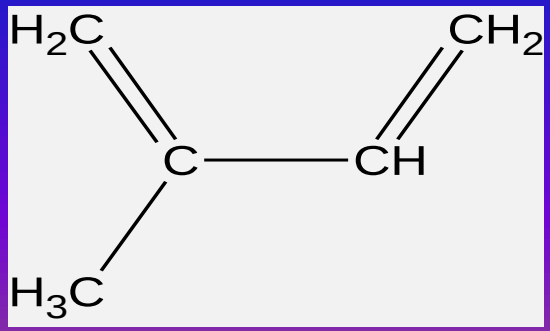



Structure of carotenoids



Groups on xantophylls: hydroxyl, epoxy, aldehyde and ketone

isoprene unit = basic structure

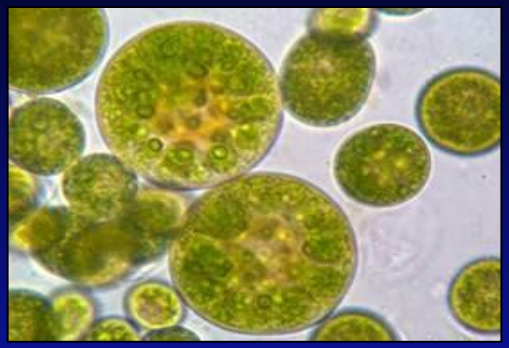


CURRENT HYPOTHESIS

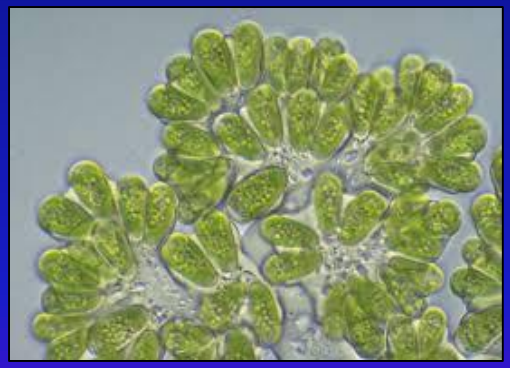
Evaluation of carotenoid production from microalgae and their role in food, feed and nutracetuical applications

Microalgae species

- *Haematococcus*

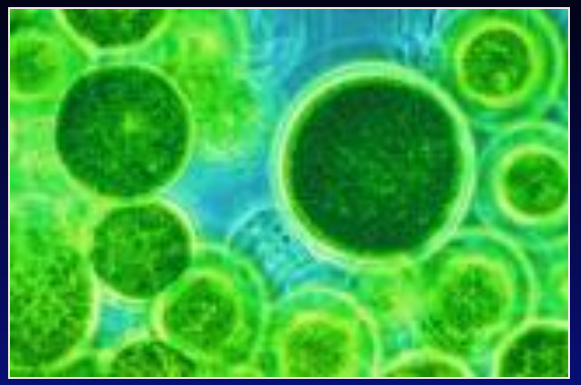


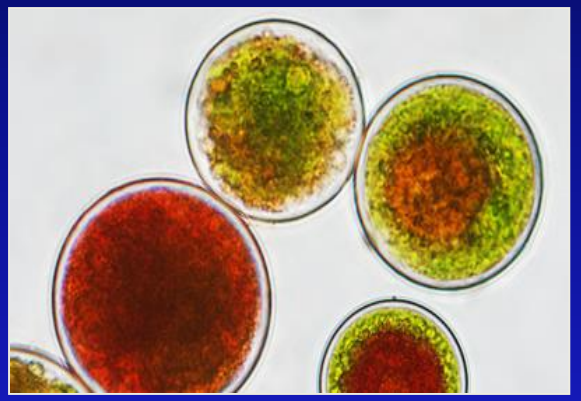
- *Botryococcus*

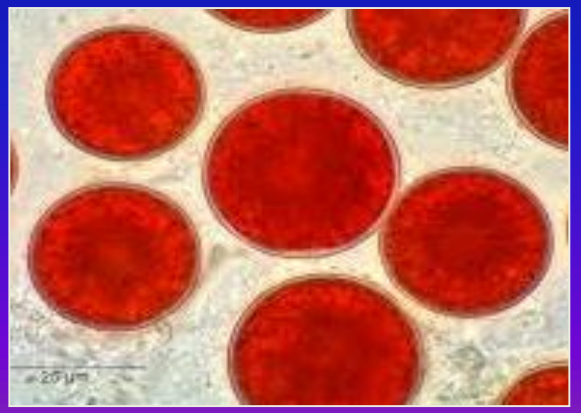


Haematococcus pluvialis

- **Fresh water, unicellular, biflagellate motile green alga**
- **Astaxanthin is accumulated (2-3% of the dry weight) under unfavorable culture conditions such as high light, nutrient deprivation (nitrogen or phosphate deficiency) and salt stress etc.**
- **High light and salt stress in encysted cells lead to astaxanthin accumulation**
- **Accumulated in cytoplasm (lipid globules) - diesters(25%), monoesters(70%) and free form (5%)**



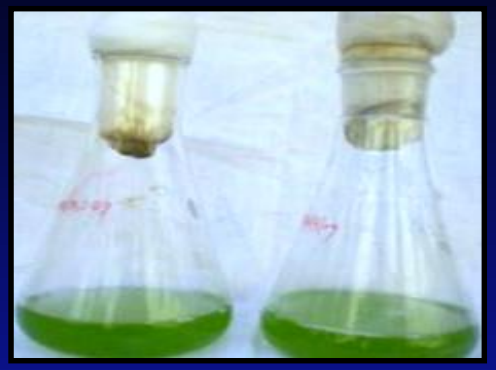




Zhang et al., 2016; Xi et al., 2016; Ranga Rao et al., 2014

Carotenoid production

- The maximum biomass yield 3.3 (g/L), total carotenoid content (2.9%) and astaxanthin content (2.5%) were obtained in sodium nitrogen treated culture
- Potassium chloride treated culture obtained the maximum biomass yield (2.5 g/L), total caroteniod (2.3%) and astaxanthin content (1.95%) when compared to control culture (without stress)
- The maximum biomass yield (2.9 g/L), total carotenoid (2.6% w/w) and astaxanthin content (2.2%) were obtained in ammonium carbonate
- The maximum extractability of caroteniods were found in palm oil and olive oil
- Among the solvents, ethylacetate, isopropyl alcohol: hexane followed by acetone could extract more caroteniods from *Haematococcus* biomass



Identification and quantification of carotenoids

S. No	Carotenoids	*H. pluvialis* (%)
1	Neoxanthin	0.9 ± 0.05
2	Violaxanthin	0.3 ± 0.12
3	astaxanthin	3.8 ± 0.25
4	Lutein	1.4 ± 0.20
5	Zeaxanthin	4.2 ± 0.38
6	Unidentified	2.8 ± 0.16
7	β-Cryptoxanthin	5.3 ± 0.25
8	β-Carotene	1.7 ± 0.98
AE	Astaxathin esters	77.58 ± 3.12

S. No	Identification of carotenoids	Formula	Fragmentation Mass
1a	Violaxanthin	$C_{40}H_{56}O_4$	601.60
1b	Neoxanthin	$C_{40}H_{56}O_4$	601.60
2	Astaxanthin	$C_{40}H_{52}O_4$	596.62
3	Lutein	$C_{40}H_{56}O_2$	569.54
4	Zeaxanthin	$C_{40}H_{56}O_2$	569.54
5	α-Carotene	$C_{40}H_{56}$	537.51
6	β-Carotene	$C_{40}H_{56}$	537.51
7	Echinenone	$C_{40}H_{54}O$	551.53
9	Antheraxanthin	$C_{40}H_{56}O_3$	585.51

Ranga Rao et al., (2009) J. Microbiol. Biotechnol. 19(11): 1333-1341

Botryococcus braunii

- It is a green colonial microalga belonging to family *Chlorophyceae*
- Mainly known for the production of hydrocarbons, polysaccharides and carotenoids etc.,
- Grouped into three different chemical races A, B and L depending on the type of hydrocarbons synthesized
- Race A, B and Race L produced more carotenoids such as β-carotene, echinenone, 3-(OH) echinenone, canthaxanthin, lutein, violaxanthin, loroxanthin and neoxanthin

Ranga Rao et al., 2012; 2007; 2006;

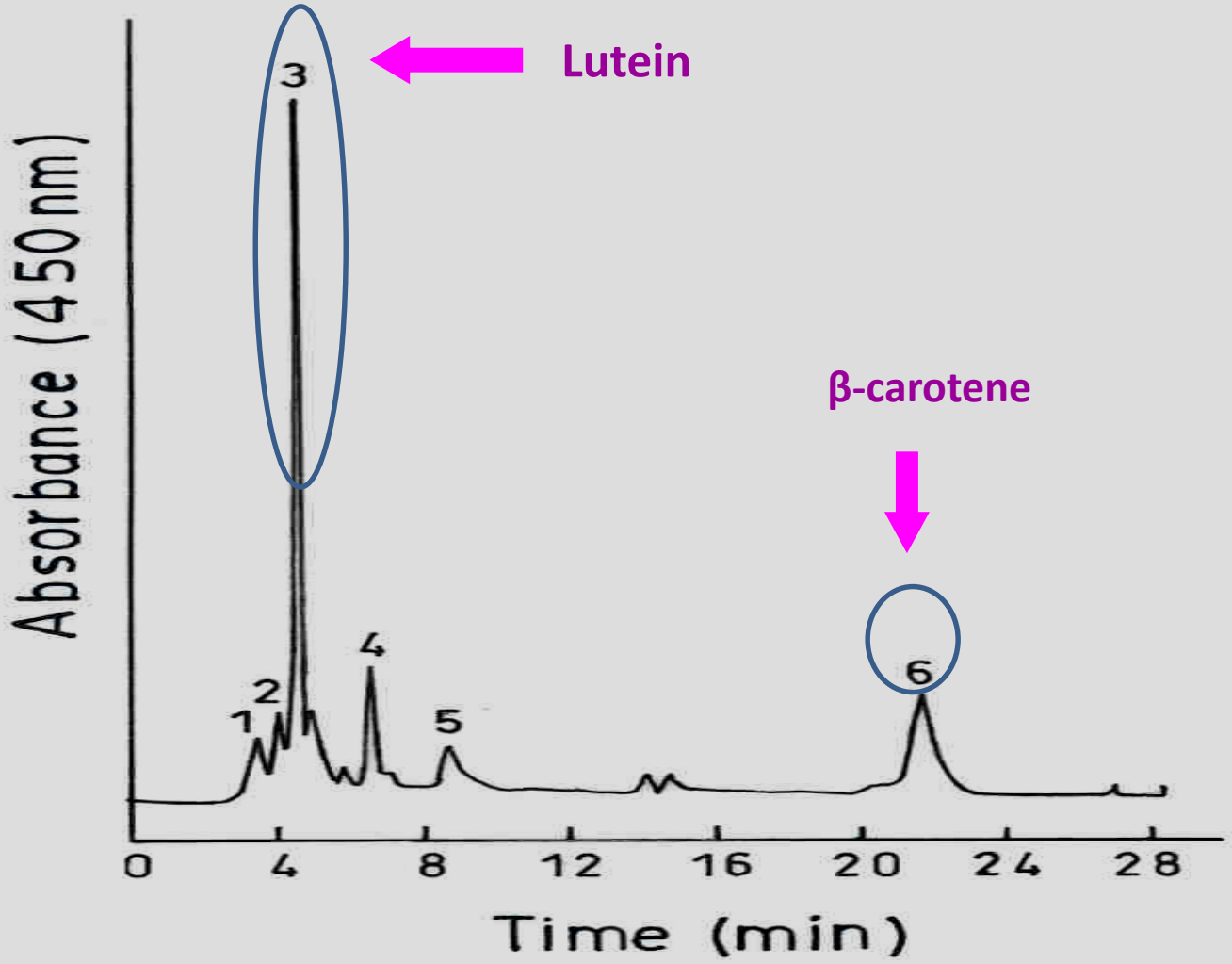
Influence of media on biomass and carotenoid production in *B. braunii* culture.

Media	Biomass yield ($g\ L^{-1}$)	Total carotenoid ($mg\ g^{-1}$)
Chu13	**2.7 ± 0.16**	**1.7 ± 0.07**
2	**2.9 ± 0.03**	**1.8 ± 0.05**
3	**3.1 ± 0.07**	**2.3 ± 0.05**
4	**3.0 ± 0.27**	**2.0 ± 0.23**
Z8	**3.1 ± 0.09**	**1.8 ± 0.02**
2	**3.2 ± 0.03**	**2.7 ± 0.08**
3	**3.0 ± 0.01**	**2.3 ± 0.06**
4	**3.2 ± 0.02**	**2.5 ± 0.02**
BBM	**2.4 ± 0.04**	**1.9 ± 0.02**
2	**2.4 ± 0.08**	**1.6 ± 0.03**
3	**2.9 ± 0.02**	**2.2 ± 0.05**
4	**2.1 ± 0.02**	**1.4 ± 0.07**
BG11	**3.2 ± 0.05**	**2.5 ± 0.05**
2	**3.6 ± 0.02**	**2.8 ± 0.03**
3	**3.3 ± 0.04**	**2.3 ± 0.08**
4	**3.1 ± 0.02**	**2.3 ± 0.07**

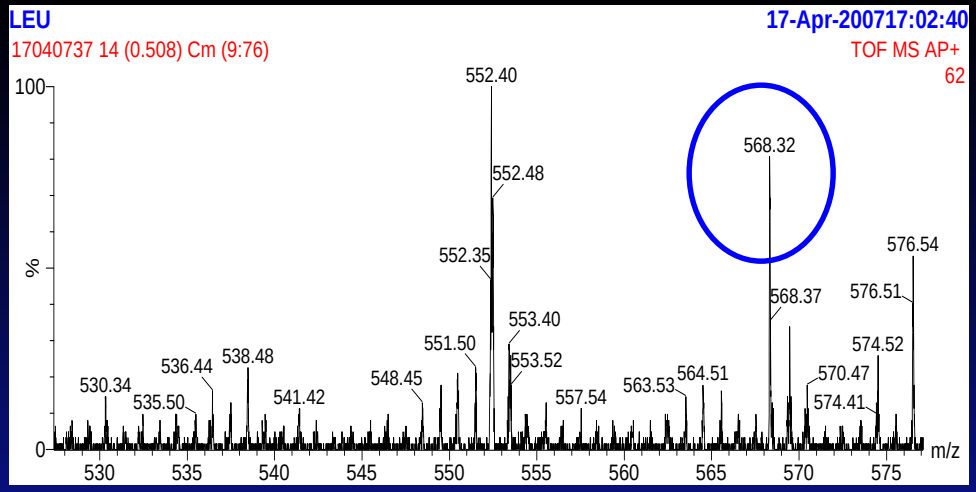
1=Control, 2=0.1% NaCl, 3=0.1% NaCl + 0.1% NaHCO3, 4=0.1% NaCl + 4mM Na Acetate

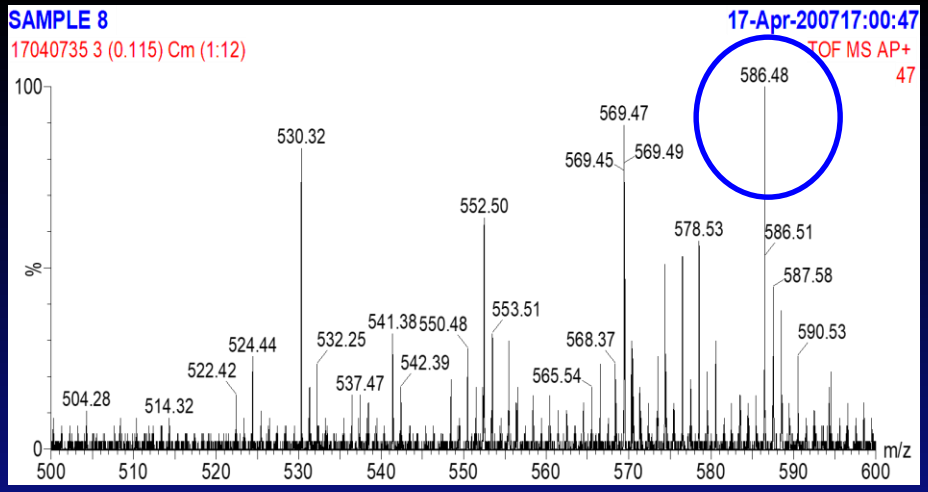
Ranga Rao et al., (2010) Int. J. Biomed. Pharm. Sci. (2): 87-92

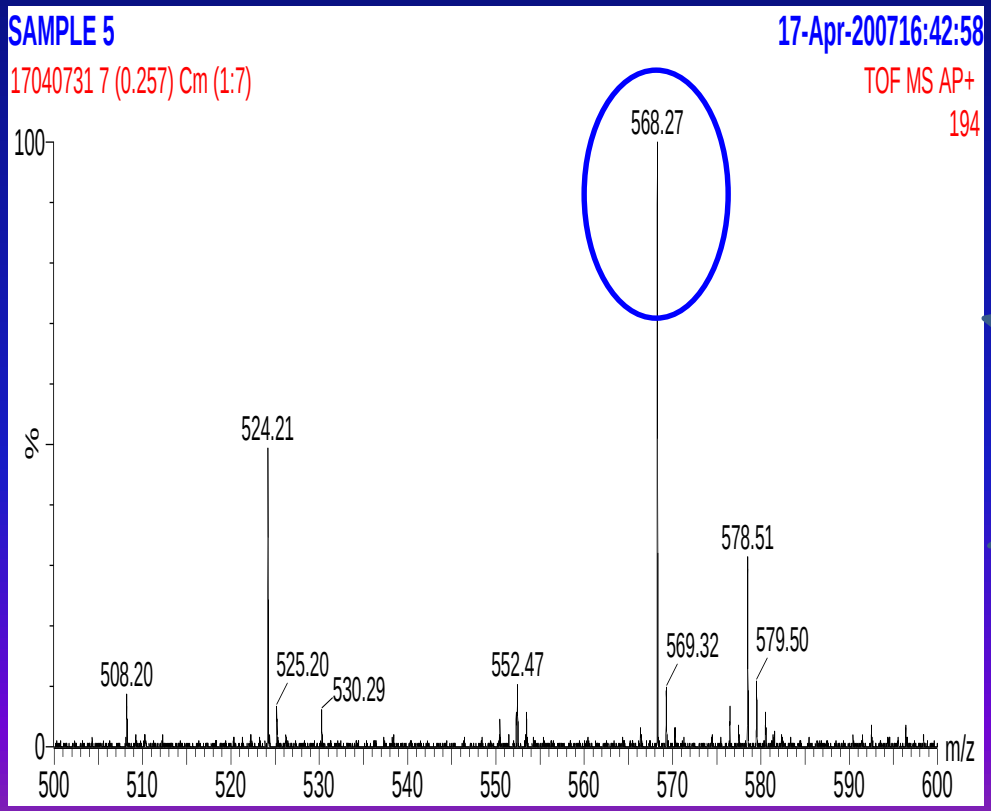
Identification of carotenoids by HPLC



Ranga Rao et al., (2010) Int. J. Biomed. Pharm. Sci. (2): 87-92







Identification of lutein by LC-MS

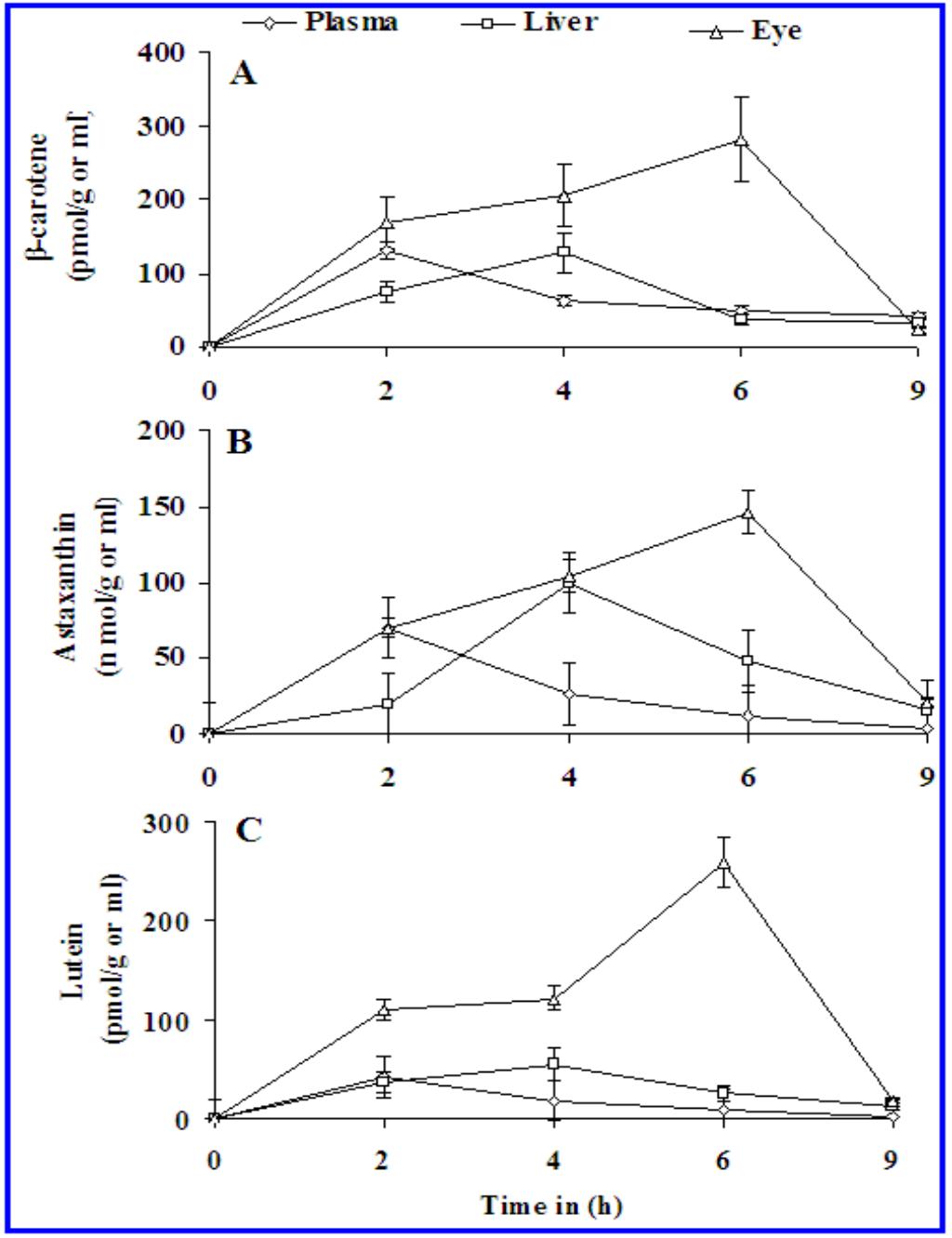
Ranga Rao et al., (2010) Int. J. Biomed. Pharm. Sci. (2): 87-92

Identification of carotenoids from *B. braunii* by LC- (APCI)-MS

S. No	Carotenoid	Formula	Molecular mass	Major ion	Identity
1	Violoxanthin	$C_{40}H_{56}0_4$	600.42	586.5	$[M\text{-}H_20+4H]^+$
2	Astaxanthin	$C_{40}H_{52}0_4$	596.38	598.3	$[M+2H]^+$
3	Lutein	$C_{40}H_{56}0_2$	568.43	568.2	$[M]^+$
4	Zeaxanthin	$C_{40}H_{56}0_2$	568.43	569.2	$[M+H]^+$
5	α-carotene	$C_{40}H_{56}$	536.44	537.5	$[M+H]^+$
6	β-carotene	$C_{40}H_{56}$	536.44	538.5	$[M+2H]^+$
7	β-cryptoxanthin	$C_{40}H_{56}0$	552.43	553.5	$[M+H]^+$
8	Antheraxanthin	$C_{40}H_{56}0_3$	584.42	586.4	$[M+2H]^+$

Ranga Rao et al., (2010) Int. J. Biomed. Pharm. Sci. (2): 87-92

Carotenoids in plasma, liver and eye of rats after feeding *S. platensis* (A), *H. pluvialis* (B), and *B. braunii* (C) as source of β-carotene Astaxanthin, and lutein



Ranga Rao et al., (2010) J. Agri. Food Chem. 58 (15), 8553-8559

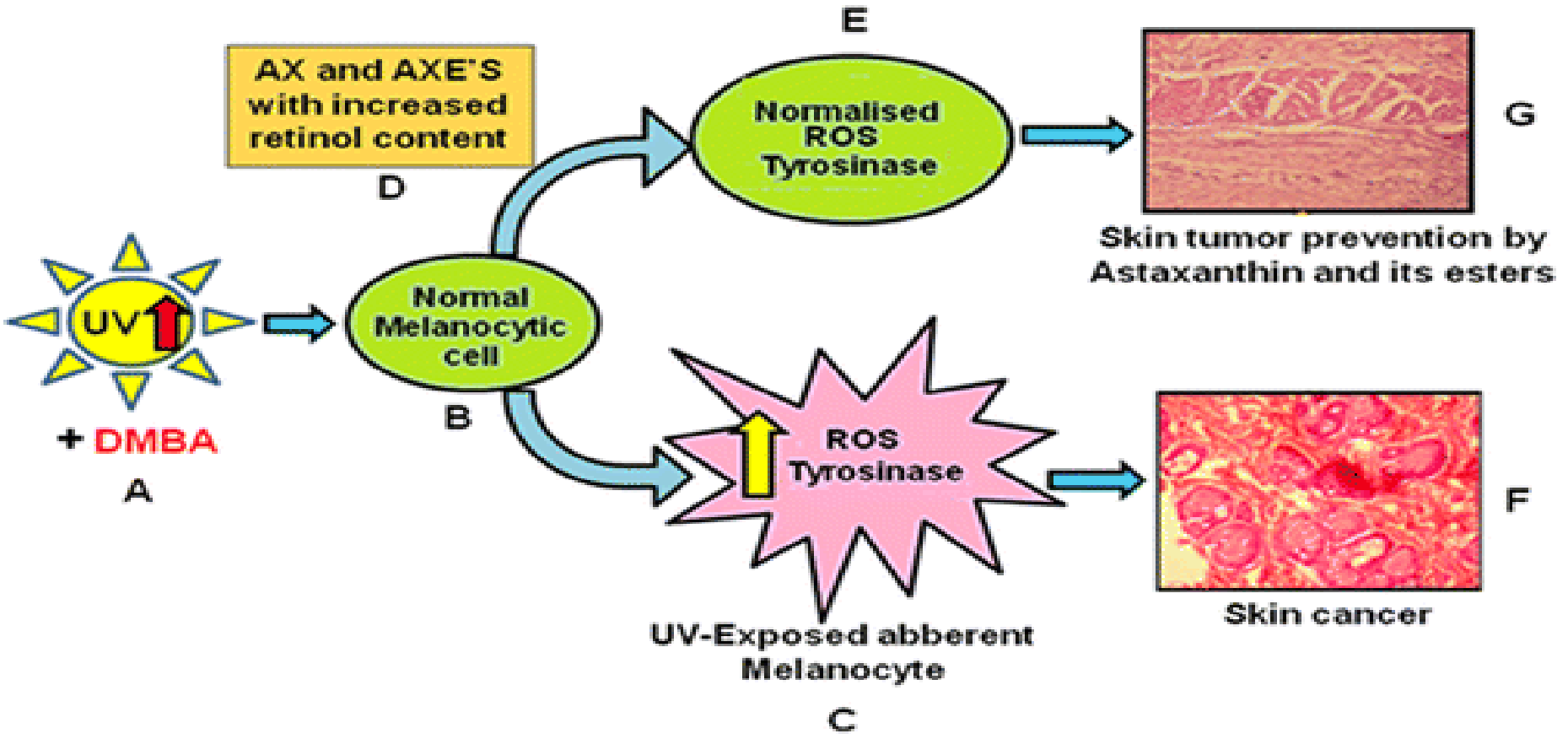
Antioxidant enzymes in plasma and liver of rats after feeding of *S. platensis*, *H. pluvialis* and *B. braunii* biomass as source of β-carotene, astaxanthin and lutein

Time in (h)	*S. platensis* (U/mg protein)	*H. pluvialis* (U/mg protein)	*B. bruanii* (U/mg protein)
Catalase			
0	106.85 ± 3.59^{a}	106.85 ± 3.59^{a}	106.85 ± 3.59^{a}
2	231.31 ± 8.93^{b}	250.10 ± 12.03^{b}	215.68 ± 5.67^{b}
4	221.67 ± 5.95^{b}	243.58 ± 8.03^{b}	198.99 ± 8.07^{c}
6	176.06 ± 3.42^{c}	199.23 ± 5.47^{c}	185.03 ± 11.01^{c}
9	140.16 ± 2.73^{d}	178.55 ± 15.42^{c}	168.17 ±12.17^{d}
SOD			
0	7.57 ± 1.21^{c}	7.57 ± 1.21^{c}	7.57 ± 1.21^{c}
2	12.89 ± 2.65^{a}	12.12 ± 3.25^{a}	11.09 ± 1.97^{a}
4	8.48 ± 1.65^{b}	11.14 ± 6.32^{a}	10.35 ± 2.9^{a}
6	9.46 ± 0.35^{b}	10.01 ± 0.54^{a}	10.5 ± 2.2^{a}
9	11.59 ± 2.72^{a}	9.09 ± 2.51^{b}	9.86 ± 0.74^{b}
Peroxidase			
0	7.35 ± 1.55^{c}	7.35 ± 1.55^{c}	7.35 ± 1.55^{c}
2	14.95 ± 1.76^{a}	15.05 ± 0.92^{a}	13.84 ±1.0^{a}
4	14.28 ± 3.43^{a}	12.86 ± 0.61^{b}	12.27 ± 3.4^{b}
6	12.53 ± 0.95^{b}	10.51 ± 0.61^{b}	10.53 ± 0.91^{b}
9	10.34 ± 1.99^{b}	9.95 ± 1.14^{b}	9.79 ± 0.30^{b}

Time in (h)	*S. platensis* (U/mg protein)	*H. pluvialis* (U/mg protein)	*B. bruanii* (U/mg protein)
Catalase			
0	251.52 ± 2.10^{c}	251.52 ± 2.10^{c}	251.52 ± 2.10^{c}
2	358.46 ± 4.40^{a}	336.67± 4.51^{b}	328.11 ± 6.36^{b}
4	382.48 ± 6.73^{a}	383.08 ± 1.81^{a}	371.29 ± 5.68^{a}
6	370.52 ± 5.26^{a}	374.45 ± 4.34^{a}	356.83 ± 2.15^{a}
9	331.76 ± 7.29^{b}	366.44 ± 4.34^{a}	344.72 ± 6.34^{a}
SOD			
0	10.34 ± 0.54^{c}	10.34 ± 0.54^{c}	10.34 ± 0.54^{c}
2	13.41 ± 4.75^{b}	13.80 ± 4.47^{b}	12.91± 2.58^{a}
4	17.21± 1.19^{a}	17.19 ± 3.87^{a}	16.30 ± 4.54^{a}
6	15.03 ± 2.91^{a}	16.29 ± 1.21^{a}	15.64 ± 1.81^{a}
9	14.46 ± 2.96^{b}	15.65 ± 0.85^{a}	13.92± 0.78^{b}
Peroxidase			
0	7.44 ± 2.64^{c}	7.44 ± 2.64^{c}	7.44 ± 2.64^{c}
2	9.22 ± 4.39^{c}	10.11±1.47c	8.42 ± 2.83^{c}
4	15.94 ± 2.84^{a}	16.28 ± 2.25^{a}	13.34 ± 4.34a
6	14.94 ± 6.33^{a}	14.63 ± 3.99^{a}	12.6 ± 5.95a
9	11.38 ± 5.2^{b}	11.32 ± 2.37^{b}	9.43 ± 1.54^{c}

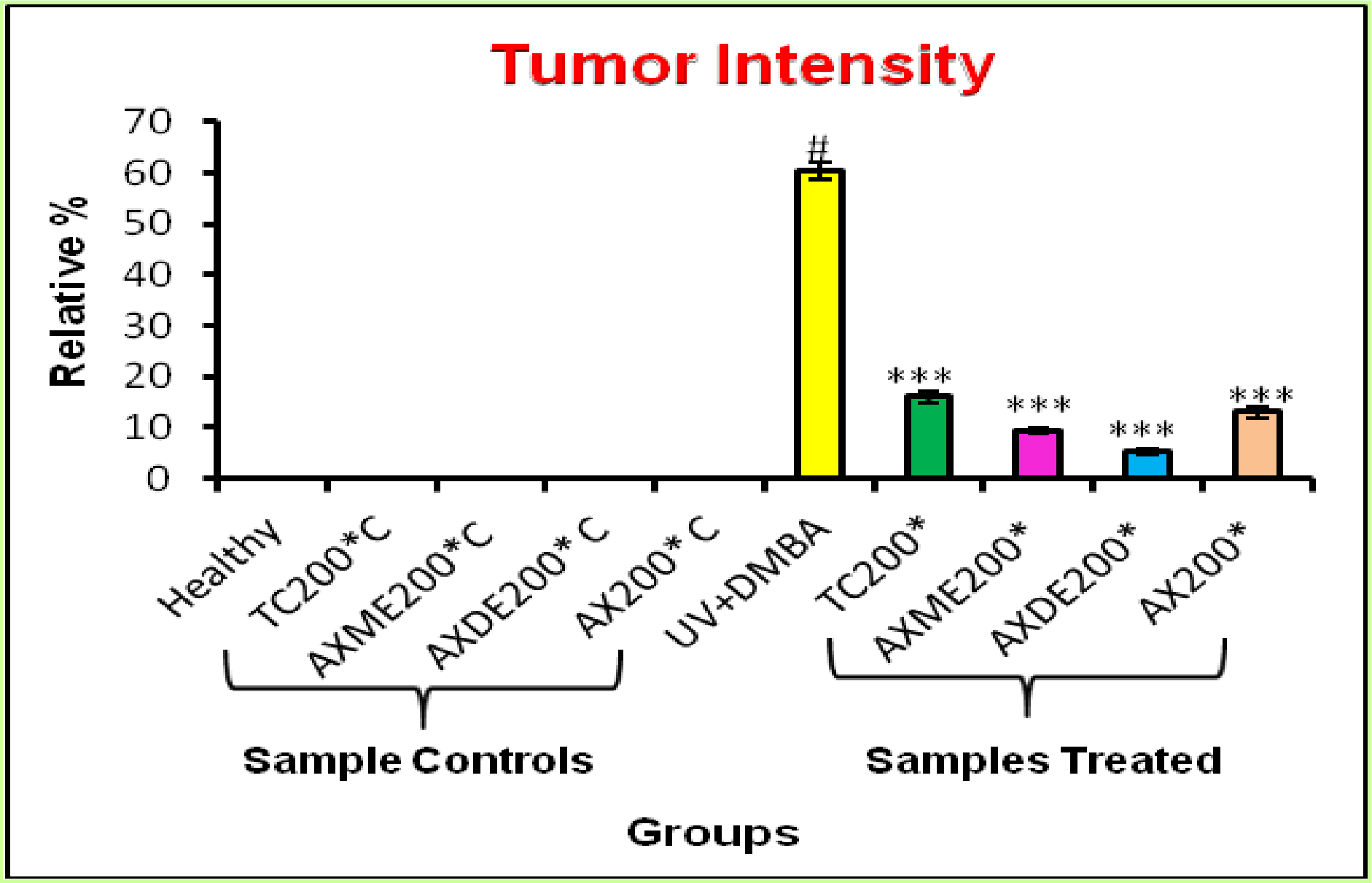
Ranga Rao et al., (2010) J. Agri. Food Chem. 58 (15), 8553-8559

The Protective Role of carotenoids on UV-DMBA induced Skin Carcinogenesis Rats: Possible Mechanism of Action



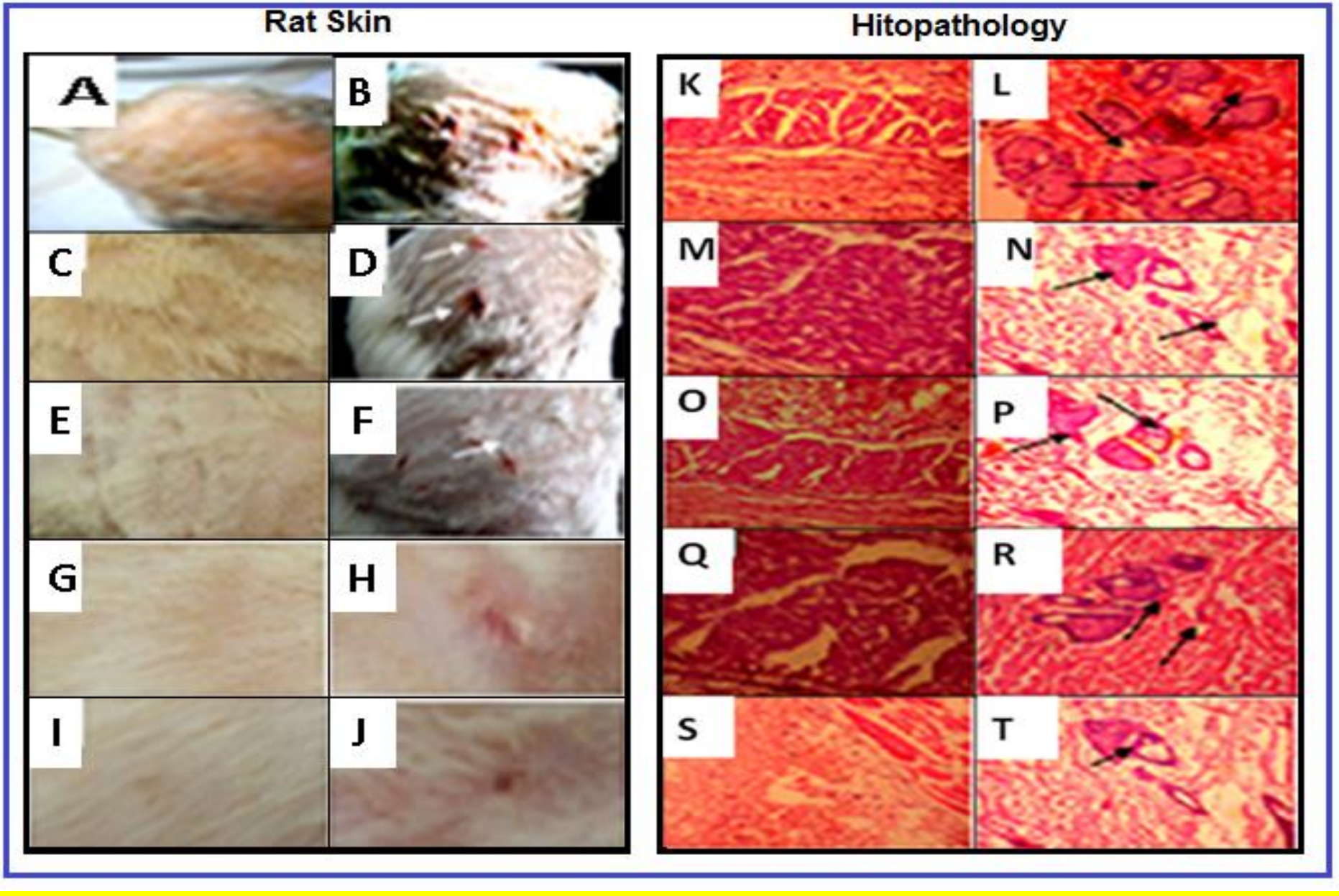
Ranga Rao et al., (2013) J. Agri. Food Chem., 61 (16), 3842-3851

Tumor intensity



Ranga Rao et al., (2013) J. Agri. Food Chem., 61 (16), 3842-3851

Histopathology



CONCLUSION

The current results indicate that the microalgae species can be exploited for carotenoid production, further these compounds can be used for the treating vitamin A deficiency and malnutrition problems which are major global public health issues.

Thank you