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Pancreatic-Lipase Inhibitory and Radical Scavenging Activities of Antheraxanthin-Di-Acetate

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Abstract

Aim: To study pancreatic lipase inhibitory and antioxidant activities of antheraxanthin- di-acetate (ADA) synthesized from antheraxanthin (AX) which is extracted from orange juice. **Methods:** Extraction of antheraxanthin from orange juice, purification by TLC and synthesis of ADA from the extracted AX and analysis of radical scavenging activity by 2-Deoxy D-ribose assay and pancreatic lipase inhibitory activity by PNPP method. **Results:** The yield of AX was 2.36mg/g of orange pulp and ADA was 0.03mg/ml. Wavelength scan of AX and ADA showed λ max of 510nm and 440 nm respectively. The percentage of inhibition of hydroxyl radicals, in standard BHA was 88.88%. Whereas, AX showed inhibition of 56.25% and ADA inhibited by 74.35% respectively. Percentage of Pancreatic lipase inhibitory activity of AX and ADA was about 12.53 and 69.57 in 100µl of sample respectively. **Conclusion:** ADA has better antioxidant and lipase inhibitory activities when compared with AX. Hence with further investigation, it can be developed as a potent anti-obese drug.

Keywords
Antheraxanthin, Antheraxanthin-di- acetate, Pancreatic lipase, Para-nitro phenyl palmitate

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INTRODUCTION

Obesity is one of the most prevailing nutritional disorders affecting millions of people and shortening lifespan of an individual worldwide, which may be the result of sedentary lifestyle, less of physical activities and overconsumption of fat containing foods[1]. Obesity in turn leads to various associated diseases like type 2 diabetes, hypertension, coronary heart disease, cancer, respiratory complications and osteoarthritis[2]

Major goals in the treatment of obesity include not only weight reduction, but also a reduction of obesity-related complications such as insulin-resistance, hyperlipidemia and cardiovascular diseases. The most commonly used method of

treating obesity is calorie restriction (CR) combined with increased physical activity; however, long-term effects of dietary interventions are frequently disappointing. Apart from bariatric surgery, which is effective but also the most expensive, efficacy of other medical therapies is limited and therefore there is a constant need for novel, non-invasive methods for the treatment of obesity and related complications[3].

Lipid metabolism involves numerous enzymes in digestion, absorption, synthesis, mobilisation, storage and oxidation. Shi and Burn have reported that lipid metabolic enzymes are the emerging drug targets for the treatment of obesity[4]. Pancreatic lipase is one of the enzymes, which hydrolysetriacylglycerols into free fatty acids and glycerols. These are absorbed by cells and excess of fatty acids are stored in adipose tissue leading to obesity. Hence inhibition of pancreatic lipase can control obesity[5].

In market, orlistat a Pancreatic and gastric lipase inhibitor is available which reduces fat malabsorption and net energy intake. Dosage of orlistat is 120 mg before meals three times a day is prescribed but the common side effects include oily spotting, flatus with discharge, fecal urgency, oily evacuation, increased defecation and fecal incontinence. Long term usage may result in cardio vascular events and stroke[6]. Hence, there is an urgent need for safe antiobese drugs especially derived from the natural source.

In recent years, natural carotenoids have attracted a great deal of attention because of their diverse and beneficial bioactivities, such as antioxidant, anticancer, anti-inflammatory and anti-obesity activities[7]. Fucoxanthin, an extensively studied carotenoid from marine brown algae, was reported to have remarkable antiobesity properties, including decreased body weight gain and improved lipid metabolism in both obese mice, [8, 9]and high-fat-diet–induced obese mice[10, 11]. The green algal carotenoid Siphonaxanthin is shown to inhibit adipogenesis in 3T3-L1 preadipocytes and the accumulation of lipids in White Adipose Tissue of KK-Ay Mice[12].

Antheraxanthinis a mono epoxy carotenoid obtained by the removal of one epoxy group from violoxanthin by violoxanthinde-epoxidase. It is a light harvesting pigment having a major role in photosynthesis. It is obtained from red algae, euglenoids and plants[14]. There is a sparse knowledge about the physiological aspects of antheraxanthin. Hence, in this study we have made an attempt to understand lipase inhibitory and antioxidant property of antheraxanthin and its derivative antheraxanthin di acetate extracted from orange juice.

MATERIALS AND METHODS

Chemicals

Lipase from porcine pancrease and 4-Nitrophenyl palmitate was obtained by sigma Aldrich Co. USA. & other chemicals viz hexane, methanol, diethylether, silica gel G, ethanol, $FeCl_3$, EDTA, H_2O_2, 2-Deoxy D-ribose, chloroform, isopropanol, ascorbic acid, pyridine, acetic anhydride, ethyl acetate, sulphuric acid, potassium dihydrogen phosphate, butyl-hydroxy anisole(BHA), HCL, dipotassium hydrogen phosphate, n-butanol were of analytical grade obtained from SRL, Himedia and Merck, Co.

Oranges were purchased from Hopcoms, Chamundipuram Mysuru and processed for extraction on the same day.

Extraction of antheraxanthin (AX) from oranges

300ml of orange juice was obtained by squeezing five medium sized orange weighing ≈90g each and extraction was carried out according to Melendez et al with minor modification[14]. Briefly, orange juice along with 500ml of ethanol: hexane (1:1) was poured in to separating funnel and shaken well. The coloured extract was collected, filtered and allowed to saponify in a dark brown bottle using 10% ethanolic KOH overnight in a magnetic stirrer at 1000rpm. The saponified extract was washed four times with water (4×300mL) to remove the traces of alkali and dried in a rotary evaporator at a temperature below 35^0C. The residue thus obtained was taken further for purification by TLC.

Purification of AX by preparative TLC

The extracted residue 1.23g was chromatographed on silica gel, preparative TLC plates using diethyl ether as the mobile phase. Lutein was used as internal standard to determine the location of the band corresponding to dihydroxycarotenoids. After 1h, the bands other than the bands corresponding to dihydroxycarotenoids(lutein) i.e, mono and diepoxycarotenoids were scraped [mono and diepoxy carotenoids were confirmed when these bands exposed to air turned green and blue colour respectively] and rechromatographed on aluminium TLC plates using the solvent system acetone: petroleum ether (40-60 °C) at a ratio of 3:7. The intense band obtained after the development of TLC plate was scraped and stored at -18ºC until analysis.

Synthesis of ADA from AX

Freshly distilled acetic anhydride (2ml) was added to Antheraxanthin solution (163mg in 6 ml of pyridine). Reaction mixture was stirred in magnetic stirrer for 12 hrs under reflux, then cooled and poured into ice

cold water. The resulting crystalline residue precipitate was further purified by preparative TLC to obtain red crystals (ethyl acetate/hexane 1:5)[15].

Determination of hydroxyl radical scavenging activity of ADA and AX by 2-Deoxy D-ribose assay

2-Deoxy D-ribose assay was performed to determine the hydroxyl radical scavenging activity of Antheraxanthin. The reaction mixture containing $FeCl_3$ (100µM), EDTA (104µM), H_2O_2 (1mM), 2-Deoxy D-ribose (2.8mM) were mixed with 20mM of potassium phosphate buffer, pH 7.4 with or without AX/ADA each at 100µg concentration and incubated for 1hr at 37° C. A similar assay was done with butylated hydroxyl anisole (BHA) at 100µg concentration serving as positive control following the addition of 1ml of TBA (0.5%). Finally the reaction mixture was cooled in ice and optical density was measured at 535nm. Antioxidant activity was expressed as percent inhibition of hydroxyl radical formation and calculated using the formula[16]

$$\text{Percentage of inhibition} = \frac{\text{A(blank)-A(sample)}}{\text{A(blank) X 100}}$$

Pancreatic lipase inhibitory activity of AX and ADA

The pancreatic lipase inhibitory activity of AX and ADA was assayed using 4-Nitrophenyl palmitate (PNPP) as substrate and spectrophotometrically recorded at 410nm. The substrate solution was prepared by suspending 20mg of PNPP in 10ml of isopropanol**.** The suspension was sonicated until complete dissolution of PNPP. At the time of use, this stock solution was diluted with isopropanol to concentration up to 0.5mg/ml. The porcine pancreatic lipase was dissolved in Tris HCl buffer pH 8.2 at the conc. of 5mg/ml. This suspension was centrifuged at 2000g for 5 minutes and supernatant obtained was used as enzyme source. The reaction mixture (2.4ml) contained 100mM Tris HCL buffer (pH 8.2) and 530µM substrate and was 25% isopropanol. After pre warming, the reaction mixture at 37^0C, the enzyme solution was added (0.1ml) and the incubation was continued for 20min at the same temperature. The reaction was stopped by transferring the reaction vessel to a boiling water bath for 10 minutes. After 10min of the incubation the reaction mixture was cooled at room temperature and centrifuged for 5 minutes at 1500g. Absorbance of the supernatant at 410nm was determined spectrophotometrically against blank solution[17].

One enzyme unit was defined as 1 µm of para-nitrophenol enzymatically released from substrate/minute/ml.

RESULTS AND DISCUSSION

Antheraxanthin extracted from orange juice was previously considered as isolutein or lutein epoxide. It was Melendez et al in 2015 who proved that lutein epoxides are different from cis-antheraxanthin. In the present work we have acetylated the parent molecule and converted into antheraxanthin-di-acetate and also evaluated the biological properties of both anteraxanthin and antheraxanthin-di-acetate.

The yield of AX was 0.63mg/ml and 2.36mg/g of orange pulp. ADA was synthesized from AX with a yield of 0.03mg/ml. UV-*vis* spectrophotometric analysis showed the λ_{max} of 510nm and 440 nm for AX and ADA respectively. The wavelength position of λ_{max} depends on the solvent, substitutions on the hydrocarbon backbone and the number of double bonds in the conjugated system. The spectrum of Antheraxanthin is shifted towards longer wavelength, with maximum absorbance at 510nm because of its special structure having a two hydroxyl group. The spectrum of the Antheraxanthindiacetate is shifted slightly toward shorter wavelength, with maximum absorbance at 440nm because of the substitution of the acetyl group to hydroxyl group it changes structural conformation.

Carotenoids are basically very good antioxidants. Lutein and zeaxanthin found in the macula lutea region of the eye in humans are the excellent quenchers of singlet oxygen due to its 9 conjugated double bonds[18]. Lycopene has antioxidant properties better than β-carotene and α-tocopherols[19]. Astaxanthin supplementation in rodent models had an impact on NRF gene which is the principle regulator of antioxidant response[20]. AX and ADA antioxidant nature was analysed by 2-Deoxy D-ribose assay. The percentage of inhibition of hydroxyl radicals for AX was 56.25% and for ADA was74.35%.Hence ADA has better antioxidant activity when compared to AX.

There are many phytochemicals or bioactive extracts possessing anti-pancreatic lipase activity like roots of *Aconitum carmichaeli* showing the inhibition of 12.1%. 8.2% of pancreatic lipase inhibition was shown by fruit extracts of *Luffa cylindrica*[21]. We assayed lipase inhibitory property of AX and ADA by PNPP method. AX showed the inhibition of 12.53% whereas ADA was inhibited by 69.57%. Significant

inhibition was seen by ADA and hence can be developed as a promising antiobese molecule by further studies.

Table 1

Name of the sample	Lipase activity (µM/ml/min)	% inhibition of pancreatic lipase
Control	0.115±0.005	-
AX	0.100±0.022	12.53
ADA	0.035±0.007	69.57

The inhibition of lipase activity by AX and ADA. Here control refers to the lipase activity without using inhibitor. Data are presented as average ± standard deviation (n = 3) and the anti-lipase activity was investigated at a concentration of 100 µg/mL for PPL inhibition.

Figure legends

Fig1: Maximum wavelength of extracted Antheraxanthin = 510 nm.

Based on the signature wavelength comparison, the saponified Antheraxanthin showed a maximum wavelength at 510nm confirming to be Antheraxanthin.

Fig2: The spectrum of the synthesized Antheraxanthin-di-acetate is shifted slightly toward shorter wavelengths, with maximum absorbance at 440nm.

Fig 3: % of inhibition of hydroxyl group, in standard control Butylated Hydroxy Anisole along with Antheraxanthin and Antheraxanthin-di-acetate at 100µl concentration.

Fig 1:

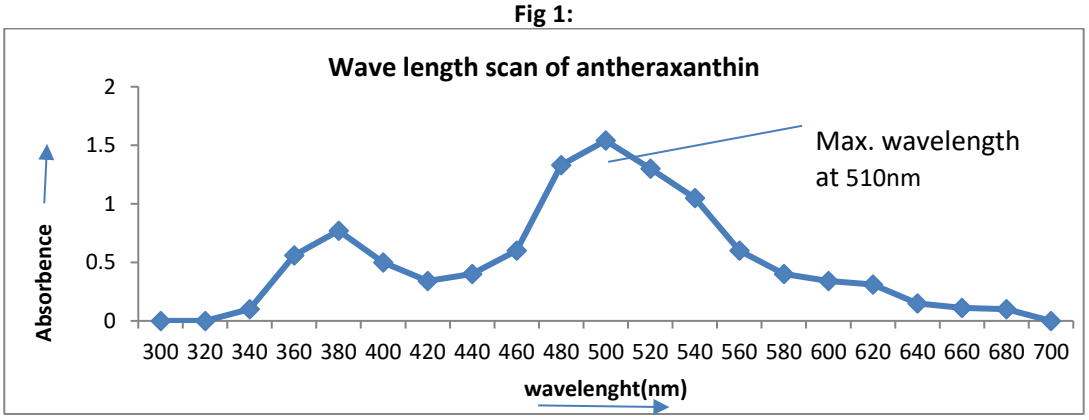


Fig 2:

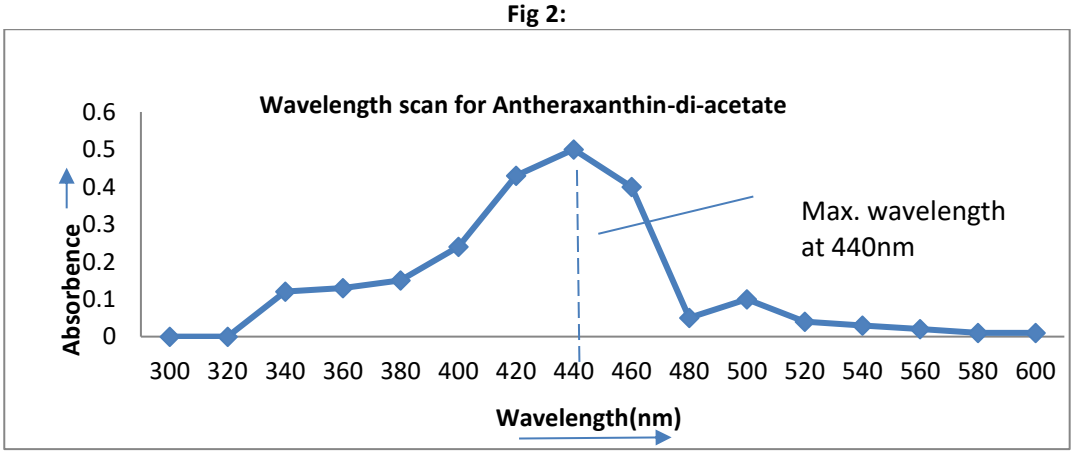
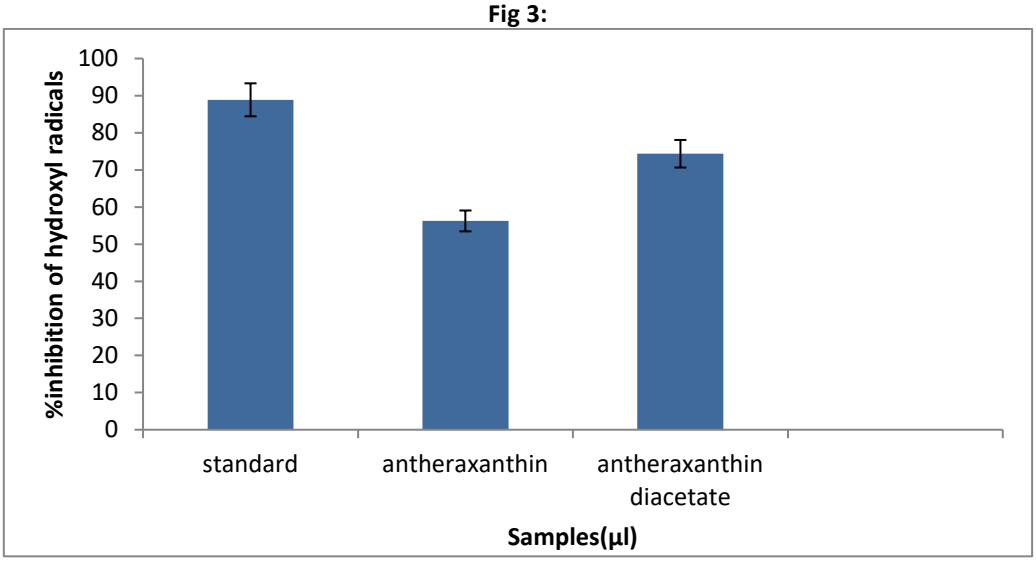


Fig 3:



CONCLUSION

The obtained Antheraxanthin and Antheraxanthin-di-acetate was stable at room temperature in the dark and anaerobic condition. Antheraxanthin-di-acetate is having better lipase inhibitory and antioxidant activities when compared with Antheraxanthin. With further investigation it can be developed as a potent anti-obese drug.

REFERENCES

[1] Francisco JT., What is happening with antiobesity drugs? Endo and Nutr, 3:101−103, (2011)
[2] Pi-Sunyer X., The medical risks of obesity. Postgrad Med, 121: 21–33, (2009)
[3] Lean M et al., ABC of obesity: Strategies for preventing obesity. Brit Medi J, 333: 959–962, (2006)
[4] Yuguang S, Burn P., lipid metabolic enzymes: emerging drug targets for the treatment of obesity**.** Natur rev, 3: 695-710, (2004)
[5] Campfield, LA, Smith FJ, Burn P., Strategies and potential molecular targets for obesity treatment. Science, 280: 1383–1387, (1998).
[6] Hanefeld M., Sachse G., The effects of orlistat on body weight and glycaemic control in overweight patients with type 2 diabetes: a randomized, placebo-controlled trial. Diabetes ObesMetab, 4: 415–423, (2002).
[7] Pangestuti R, Kim SK., Biological activities and health benefit effects of natural pigments derived from marine algae. J Funct Foods, 3: 255–66, (2011)
[8] Beppu F, Hosokawa M, Niwano Y, Miyashita K., Effects of dietary fucoxanthin on cholesterol metabolism in diabetic/obese KK-A(y) mice. Lipids Health Dis, 110-112, (2012)
[9] Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K., Fucoxanthin from edible seaweed, Undariapinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. Biochem Biophys Res Commun, 7:332-392, (2005)
[10] Jeon SM, Kim HJ, Woo MN, Lee MK, Shin YC, Park YB, Choi MS., Fucoxanthin-rich seaweed extract suppresses body weight gain and improves lipid metabolism in high-fat-fed C57BL/6J mice. Biotechnol J, 5:961–969, (2010)
[11] Woo MN, Jeon SM, Shin YC, Lee MK, Kang MA, Choi MS., Anti-obese property of fucoxanthin is partly mediated by altering lipid-regulating enzymes and uncoupling proteins of visceral adipose tissue in mice. Mol Nutr Food Res, 53:1603–1611, (2009)
[12] Zhuo-Si L, Kenji N, Eriko F, Yuki M, Takashi H, Tatsuya S., The Green Algal Carotenoid Siphonaxanthin Inhibits Adipogenesis in 3T3-L1 Preadipocytes and the Accumulation of Lipids in White Adipose Tissue of KK-Ay Mice. J Nutr, 145:490–498, (2015)
[13] Yamamoto HY., Biochemistry of the violaxanthin cycle in higher plants. Pure ApplChem, 51:64639 – 64826, (1979)
[14] Meleandez J, George B, Isabel MV, Francisco JH., Identification of Isolutein (Lutein Epoxide) as cis - Antheraxanthin in Orange juice. J Agric Food Chem, 53:9369−9373, (2005)
[15] Zhen S, Huiyuan Y., The influence of di-acetylation of hydroxyl groups on the anti-tumor-proliferation activity of lutein and zeaxanthin. Asia Pac J Clin Nutr, 16:447-452, (2007)
[16] Adam M, Patrycja T, Emilia S. Antioxidant Activity of Herb extracts from five medicinal plants from Lamiaceae. J MediPla Res, 2(11):321-330, (2018)
[17] Roselene FO, Geferson AG, Fabíola DI, Cristina GM, Adelar B, RosaneMP., Inhibition of Pancreatic Lipase and Triacylglycerol Intestinal Absorptionbya Pinhao Coat(Araucariaangustifolia) Extract Richin Condensed Tannin. Nutr, 7: 5601-5614, (2015)
[18] Krinsky NI, Landrum JT, Bone RA., Biologic mechanisms of the protective role of lutein and

zeaxanthin in the eye. Annu Rev Nutr, 23: 171–201, (2003)

[19] Rao AV, Agarwal S., Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. Nutr. Res, 19: 305–323, (1999)

[20] Hybertson BM, Gao B, Bose SK, McCord JM., Oxidative stress in health and disease: The therapeutic potential of Nrf2 activation. Mol Aspects Med, 32:234–246, (2011)

[21] Changhyun R UheeJJ., Screening of Crude Plant Extracts with Anti-Obesity Activity MolSci, 13(2): 1710–1717, (2012).