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Research Article

Qualitative Evaluation of Carotenoids Profile in Three *Polygonum* Species by HPTLC.

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Abstract

The carotenoids profile of three *Polygonum* species (*P. chinense, P. glabrum* and *P. barbatum*) was determined by HPTLC method. The methanol extracts of *P. chinense, P. glabrum* and *P. barbatum* species whole-plant samples shows 10, 7 and 5 compounds, respectively, and was compared with cryptoxanthin and zeaxanthin standards. Among the compounds, 4, 2 and 3 compound in each sample, respectively, was identified as carotenoids while others were unknown. One carotenoid compound each from *P. glabrum* and *P. barbatum* showing same peak R_f values (0.48). All other compounds detected in the three *Polygonum* species showed no similarities in their R_f values. The nature and number of carotenoid compounds in these three *Polygonum* species are varied.

Keywords: Carotenoids, HPTLC, Methanol extracts, *Polygonum barbatum*, *Polygonum chinense*, *Polygonum glabra*.

Introduction

Carotenoids are tetraterpenoid organic pigments that occur naturally in the chloroplasts and chromoplasts of plants. Most of the carotenoids found in foods of people consume have antioxidant activity. Carotenoids are efficient free-radical scavengers and they enhance the vertebrate immune system. People consuming diets rich in carotenoids from natural foods have lower mortality from a number of chronic illnesses (Diplockl *et al*., 1998) and they have vitamin-A activity and can also act as antioxidants (Sindhu *et al*., 2010; Gun-Ae Yoon *et al*., 2012). The present study is aimed to evaluate the carotenoid compound profile in the whole-plant samples of three *Polygonum* species – *P. chinense, P. glabrum and P. barbatum*.

Materials and Methods

Study area

The test plant of three *Polygonum* species were collected during 2009 from Tirunelveli (*Polygonum chinense* Linn.) and Thoothukudi (*Polygonum glabrum* Willd. and *Polygonum barbatum* Linn.) districts of Tamil Nadu, India.

Polygonum species selected

The three species of *Polygonum* belongs to Polygonaceae were identified as *P. chinense, P. glabrum* and *P. barbatum* based on their morphological features and compared with plant characters described in the Flora of the Presidency of Madras (Gamble, 1956), Indian Medicinal Plants (Kirtikar and Basu, 2003) in order to confirm the species identification.

Preparation of whole plant dry powder of *Polygonum* species

The three *Polygonum* species were collected and dried separately at room temperature (30°C±2°C) for about two weeks to get a constant weight. The dried plant

materials (as whole plant) were ground to powder by mechanical device and stored for further biochemical analysis.

Preparation of extract

The dried whole-plant materials of *Polygonum* samples (5g) from three species (*P. chinense, P. glabrum and P. barbatum*) were extracted separately with Methanol in Soxhlet apparatus for 3hrs. The extracts were cooled, filtered and concentrated using a vacuum flask evaporator. Finally these extracts were dissolved in 1ml methanol and centrifuged at 3000rpm for 5min. This methanol extract solution was used as test solution for HPTLC analysis.

HPTLC analysis

Methanol was uses as standard solution. Methanol extracts of *Polygonum* species (*P. chinense, P. glabrum and P. barbatum*) were subjected to HPTLC analysis to assess the presence of various carotinoid compounds.

HPTLC analysis for carotenoids

- ***Test solution***: Methanol extracts of *P. chinense, P. glabrum* and *P. barbatum*.
- ***Standard solution***: Methanol.
- ***Standard chemical***: *CRY*-Beta Cryptoxanthin (for *P. chinense*-X3 & *P. glabrum*-X4) and *ZEA*-Zeaxanthin (for *P.barbatum*-Y3) were used as reference standard compound.
- ***Mobile phase***: Acetone-Petroleum ether 60°C-80°C (30: 70).
- ***Spray reagent***: Anisaldehyde sulphuric acid reagent.

Sample loading

About 3µl of the methanol test solution and 2µl of standard solution (1mg in 1ml methanol) were loaded as 5mm band length in the 3 x 10 silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase and the plate was developed in the respective mobile phase up to 90mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured at white light, UV 254nm and UV366nm or 500nm.

Derivatization

The developed plate was sprayed with respective spray reagent and dried at 100°C in hot air oven. The plate was photo-documented at day light and UV 254nm/UV 366nm, using photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning

Before derivatization, the plate was fixed in scanner stage and scanning was done at UV 254nm/ UV 366nm/ UV 500nm. The peak table, peak display and peak densitogram were noted (Shah *et al*., 2008).

Results and Discussion

The chromatogram (Fig. 1) shows carotenoid profile of whole-plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and is compared with cryptoxanthin (for *P. chinense* and *P. glabrum* samples) and zeaxanthin (for *P. barbatum* sample) standard. Orange coloured fluorescent zones at UV 366nm, mode present in the given standard (beta-cryptoxanthin and zeaxanthin) and sample tracks observed in the chromatogram after derivatization, were confirmed the presence of carotenoids in the given standard (beta-cryptoxanthin and zeaxanthin) and may be in the sample (Fig. 1).

HPTLC analysis for carotenoid profile in the whole-plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) shows several peaks (R_f -values) of compounds (Tab. 1; Fig. 2) and compared with cryptoxanthin standard for *P. chinense* and *P. glabrum* samples and zeaxanthin standard for *P. barbatum* samples. The densitogram (Fig. 2) shows the profile of carotenoid compounds present in the whole plant methanol extract of *Polygonum* species (*P. chinense* –X3, *P. glabrum* –X4 and *P. barbatum* –Y3) and beta-

cryptoxanthin standard for *P. chinense* and *P. glabrum* samples and zeaxanthin standard for *P. barbatum* samples scanned at 366nm and 500nm, respectively.

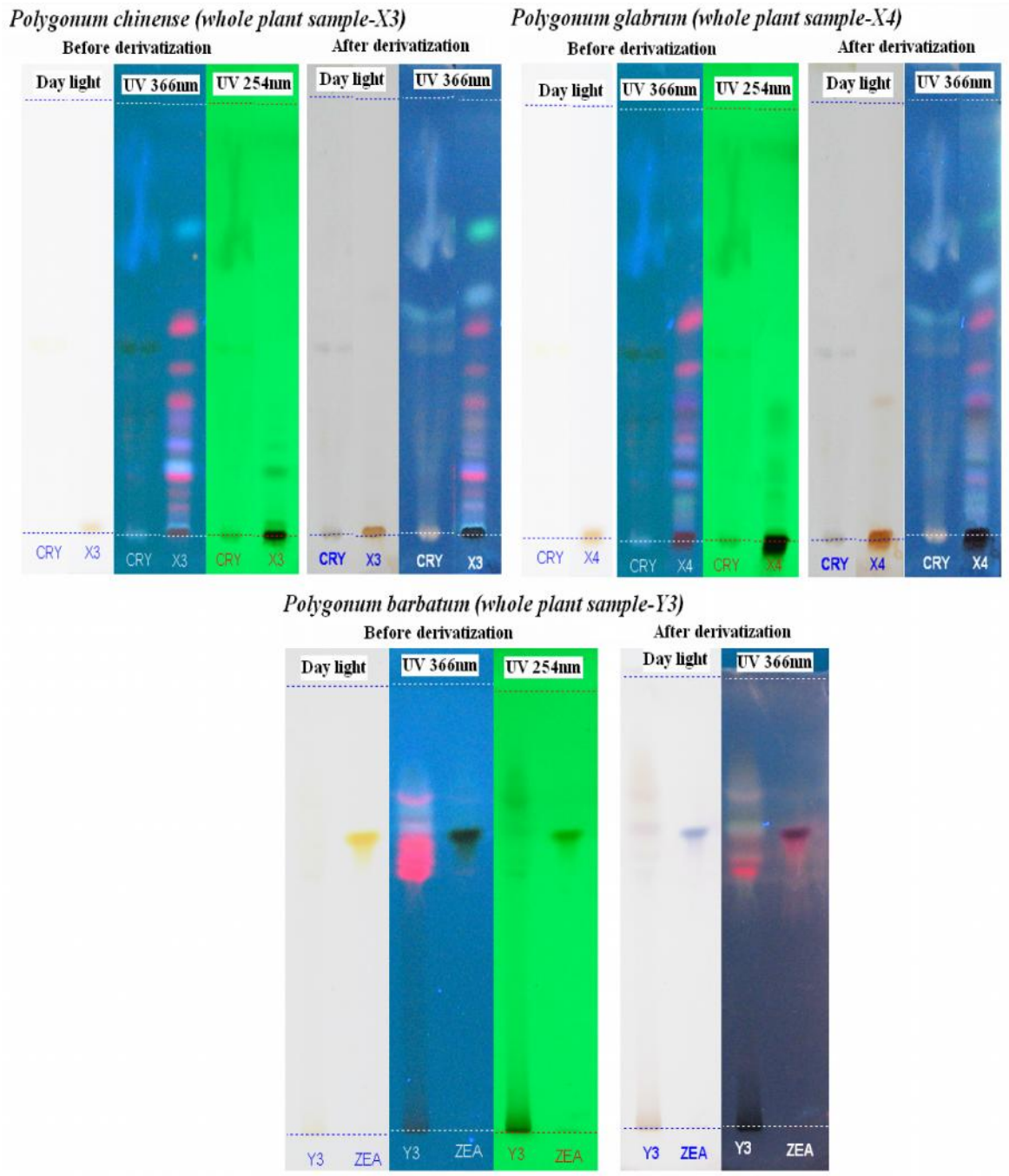

Figure 1: Chromatogram for carotenoid compounds in the whole plant methanol extract of *Polygonum* species.

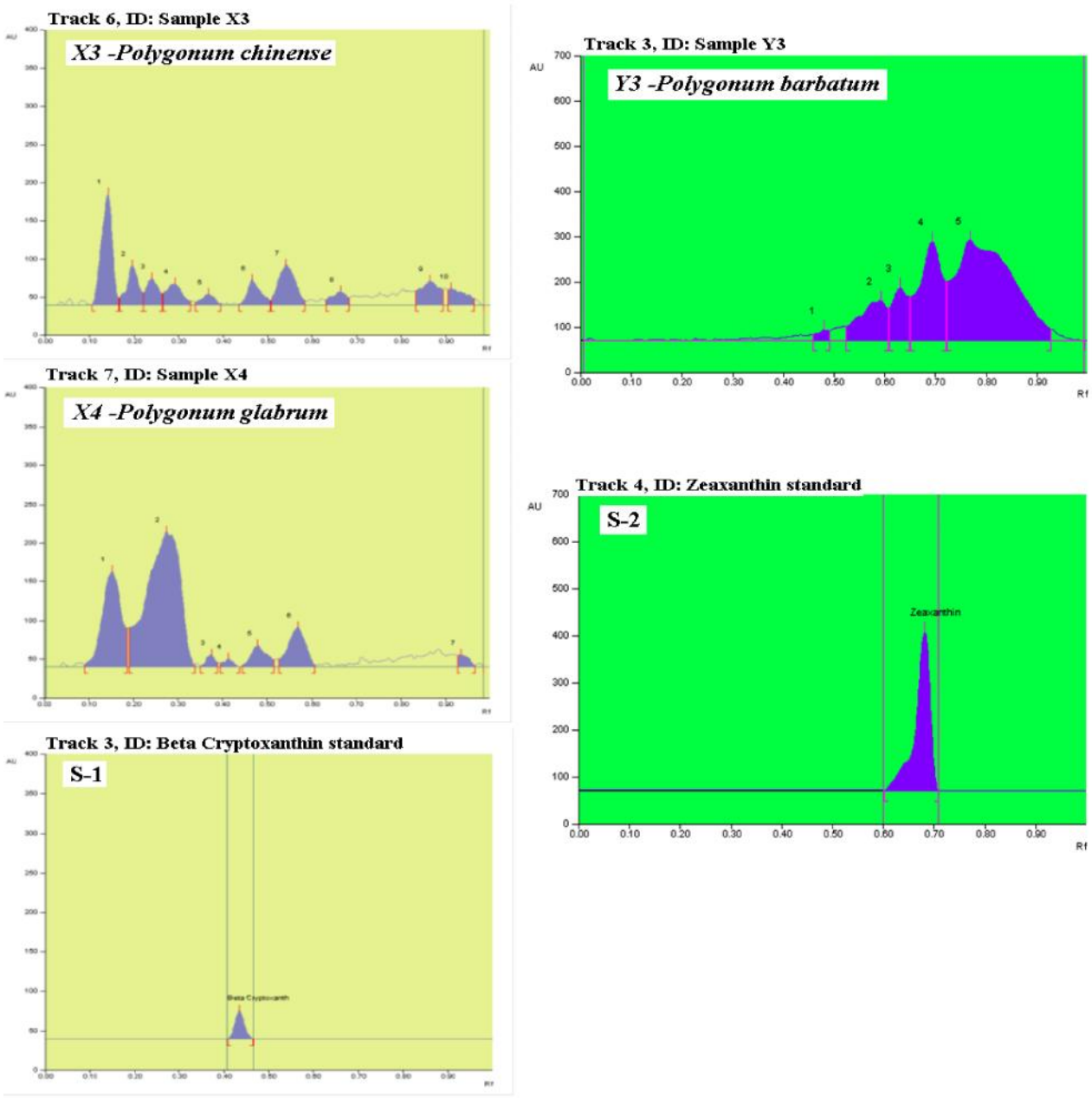

Figure 2: Densitogram showing the HPTLC analysis of carotenoid compounds in the whole plant methanolic extracts of *Polygonum* species (X3/X4/Y3); and Cryptoxanthin standard 'S-1' (for X3/X4) scanned at 366nm and Zeaxanthin standard 'S-2' (for –Y3) scanned at 500nm.

The 3D display of densitogram for carotenoid profile shows all tracks of *Polygonum* species (*P. chinense* – X3, *P. glabrum* –X4 and *P. barbatum* –Y3), and beta-cryptoxanthin for *P. chinense* (X3) and *P. glabrum* (X4); and zeaxanthin standard for *P. barbatum* (Y3) samples scanned at 366nm and 500nm, respectively (Fig. 3).

The whole-plant methanol extract of *P. chinense* (X3) shows 10 compounds with peak R_f values ranging from 0.14 to 0.91, peak height ranged from 14.0 to 145.9 and peak area ranging from 382.5 to 3278.4 as compared to cryptoxanthin standard (0.43, 59.0 and 1772.1, respectively). Among the 10 compounds detected, 4 were identified as carotenoids (No. 1, 4-6) and the others were unknown (Tab. 1-X3; Fig. 2-X3).

The whole-plant methanol extract of *P. glabrum* (X4) shows 7 compounds with varied peak R_f values (0.15-0.94), peak height (10.8-174.6) and peak area (204.7-12149.6) as compared to cryptoxanthin standard (0.43, 59.0 and 1772.1, respectively). Out of 7 compounds detected, two (No. 4 & 5) were identified as carotenoids and the others were unknown (Tab. 1-X4; Fig.2-X4).

Similarly, the whole-plant methanol extract of *P. glabrum* (Y3) shows 5 compounds (Tab. 1-Y3) with peak R_f values ranging from (0.48 to 0.77; peak height from 23.6 to 222.6 and peak area from 5326.5 to 23386.6 as compared to zeaxanthin standard (0.68, 400.2 and 15557.0, respectively) and out of 5 compounds, 4 were identified as carotenoids and other was unknown (Tab. 1-Y3; Fig. 2-Y3).

In general, the two carotenoid compounds (peak No. 5 of X4 and peak No. 1 of Y3) of *P. glabrum* and *P. barbatum* show same peak R_f values (0.48). But, the other compounds detected in the three *Polygonum* species showed no similarities (Tab. 1; Fig. 2).

Several studies suggests that carotenoids (B-carotene) effectively increases intracellular oxidative stress by increasing ROS production, etc., in many tumour cells and this effect may be accompanied by anti-tumor activity; the carotenoid may also induce cell cycle arrest and apoptosis and, even, induced the loss of tumor cell viability (Palozza *et al*., 2001; 2002; 2003). The potential of antioxidant free radical scavenging activity of *Polygonum* species was reported by several workers and this may be due to the synchronous effect of carotenoids with other compounds. In this study, the HPTLC analysis of methanol extract of *Polygonum* species make certain the presence of carotenoids and the nature and number of carotenoids present in the *Polygonum* species differ from each other.

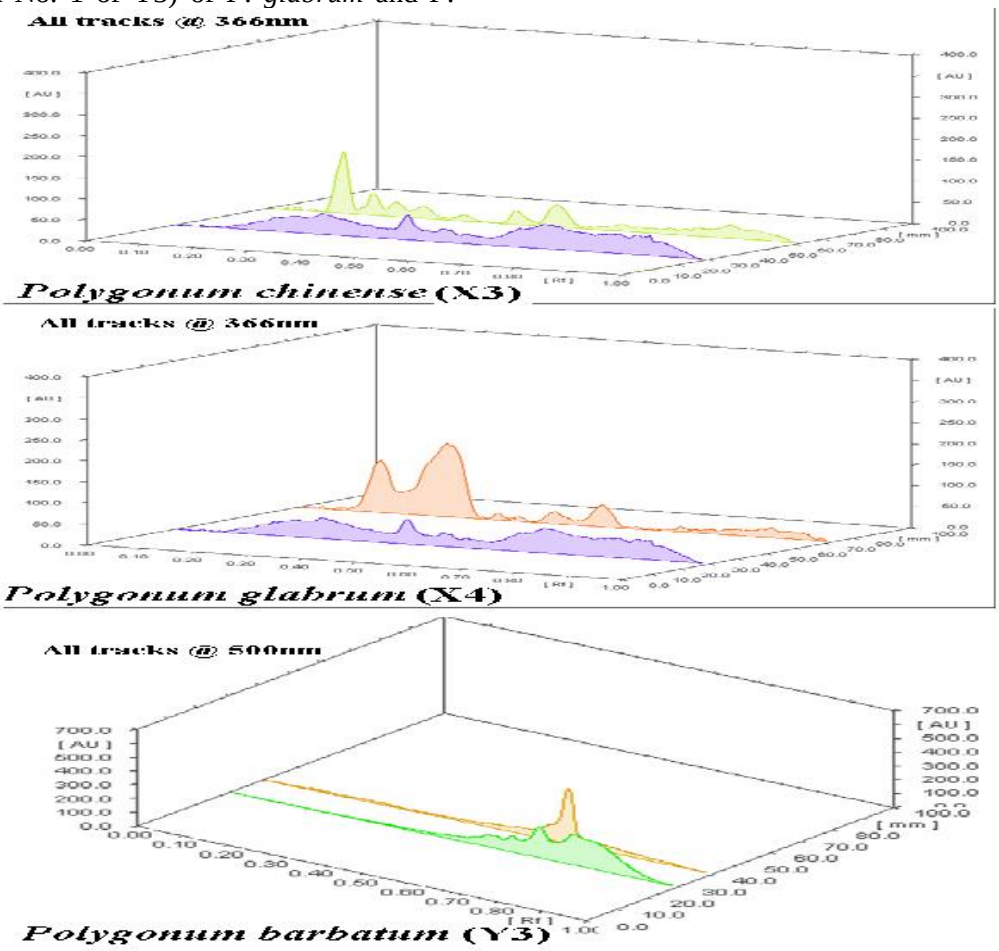

Figure 3: HPTLC densitogram 3D display of all tracks for carotenoid compounds in the whole plant methanolic extract of *Polygonum* species (X3/ X4/ Y3) and Standards (–Cryptoxanthin for X3/X4 & Zeaxanthin for Y3).

Table 1: Peak table for HPTLC analysis of carotenoid compound profile in the whole plant methanol extract of *Polygonum* species.

***P. chinense* (X3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X3	1	0.14	145.9	3278.4	Carotenoid 1
X3	2	0.20	51.2	1219.3	Unknown
X3	3	0.24	34.6	869.8	Unknown
X3	4	0.29	27.8	906.3	Carotenoid 2
X3	5	0.37	14.0	382.5	Carotenoid 3
X3	6	0.47	32.5	902.8	Carotenoid 4
X3	7	0.54	52.5	1850.1	Unknown
X3	8	0.66	17.2	538.5	Unknown
X3	9	0.86	31.1	1222.2	Unknown
X3	10	0.91	21.6	761.9	Unknown
***P. glabrum* (X4)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
X4	1	0.15	123.3	4832.2	Unknown
X4	2	0.28	174.6	12149.6	Unknown
X4	3	0.38	15.9	283.7	Unknown
X4	4	0.41	10.8	204.7	Carotenoid 1
X4	**5**	**0.48**	**28.2**	**944.6**	**Carotenoid 2**
X4	6	0.57	51.7	1827.2	Unknown
X4	7	0.94	16.1	376.2	Unknown
***P. barbatum* (Y3)**	**Peak**	**Rf**	**Height**	**Area**	**Assigned substance**
Y3	**1**	**0.48**	**23.6**	**536.5**	**Unknown**
Y3	2	0.59	88.4	4409.4	Unknown
Y3	3	0.63	116.9	3375.1	Carotenoid 1
Y3	4	0.69	219.1	8971.5	Carotenoid 2
Y3	5	0.77	222.6	23386.6	Carotenoid 3
Control-1 (X3& X4)	***1***	***0.43***	***59.0***	***1772.1***	***Cryptoxanthin standard***
Control-2 (Y3)	***1***	***0.68***	***400.2***	***15557.0***	***Zeaxanthin standard***

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