Determination of Oxalate Content in Thai Herbal Teas and Local Vegetables

การตรวจหาออกซาเลตในชาสมุนไพรและผักพื้นบ้าน

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ABSTRACT

Herbal teas and vegetables are commonly consumed because most of their phytochemicals play role in health benefits. However, they contain oxalate, which at high concentration, can cause renal stone. The aim of this study is to determine oxalate content in herbal teas and local vegetables by using a simple colorimetric method with pyrocatechol violet reagent. Eighteen commercial herbal teas and 25 local vegetables were purchased from Khon Kaen market in October 2014. *Phyllanthus amarus* Schum & Thonn. had the highest oxalate and *Ganoderma lucidum* Reishi. had the lowest oxalate content (0.0646 ± 0.0006 mg/g dry weight and 0.0056 ± 0.0004 mg/g dry weight, respectively). The local vegetables contained the highest and the lowest oxalate were *Careya sphaerica* Roxb. (0.0136 ± 0.0014 mg/g wet weight) and *Wolffia globosa* Roxb. (0.0014 ± 0.0001 mg/g wet weight), respectively. The oxalate content in these samples would be acceptable as drink and food.

บทคัดย่อ

ชาสมุนไพรและผักพื้นบ้านมีการบริโภคกันทั่วไป เพราะมีสารพฤกษเคมีที่มีประโยชน์ต่อสุขภาพ แต่อย่างไรก็ตามชาและผักที่มีออกซาเลตสูง อาจทำให้เกิดนิ่วไต ดังนั้นวัตถุประสงค์ของการศึกษาครั้งนี้คือการตรวจหาออกซาเลตในชาสมุนไพรและผักพื้นบ้านโดยใช้วิธีตรวจวัดการเปลี่ยนสีของน้ำยาไพโรแคทีคอล ไวโอเล็ต ซึ่งเป็นวิธีที่ง่าย โดยซื้อชาสมุนไพร 18 ชนิด และผักพื้นบ้าน 25 ชนิด จากตลาด ในจังหวัดขอนแก่น ในเดือนตุลาคม พ.ศ. 2557 ผลการศึกษาพบว่า ชาลูกใต้ใบ (*Phyllanthus amarus* Schum & Thonn.) มีออกซาเลตสูงที่สุด (0.0646 ± 0.0006 มิลลิกรัม / กรัมน้ำหนักแห้ง) และชาเห็ดหลินจือ (*Ganoderma lucidum* Reishi.) มีปริมาณออกซาเลตที่ต่ำที่สุด (0.0056 ± 0.0004 มิลลิกรัม / กรัมน้ำหนักแห้ง) ส่วนผักพื้นบ้านที่มีออกซาเลตสูงสุด คือ กระโดนบก (*Careya sphaerica* Roxb.) (0.0136 ± 0.0014 มิลลิกรัม/กรัมน้ำหนักสด) และออกซาเลตต่ำสุด คือ ไข่น้ำ (*Wolffia globosa* Roxb.) (0.0014 ± 0.0001 มิลลิกรัม/กรัมน้ำหนักสด) ซึ่งปริมาณออกซาเลตในตัวอย่างดังกล่าวอยู่ในระดับที่ยอมรับได้สำหรับการบริโภค

Key Words: Oxalate, Herbal tea, Local vegetable

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Introduction

Plants are major source of dietary oxalate (Franceschi VR et al., 2005). Oxalate is an antinutrient because it makes calcium unavailable for human absorption (Heaney RP et al., 1988; Weaver CM et al., 1997). Oxalate in plant foods impacts human health. High consumption of food containing oxalates has been demonstrated to be involved in urinary tract stone formation (Porena M et al., 2007) and nephrolithiasis (Khan SR et al., 2007). Calcium oxalate (CaOx) stones are now identified as the main type of urinary calculi in most countries throughout the world. Approximately 70 % of all stones were developed in the upper urinary tract and even more in male patients (Curhan GC., 2007). In some parts of the world, CaOx was found as the main component of about 90% of all kidney stones (Ansari MS et al., 2005).

There are many ways to determine oxalate in plants. Kasidas and Rose (1980) used enzymatic determination with oxalate decarboxylase, but this method may lead to elevated values oxalate due to an incomplete removal of dissolved carbon dioxide from the sample solution. Ohkawa H. (1985) used gas chromatographic technique and high performance liquid chromatography (HPLC) methods (Holloway WD et al., 1989) which are accurate and reliable for determination of oxalic acid in plant materials. Holmes RP, Kennedy M (2000) reported that capillary electrophoresis (CE) and ion chromatography (IC) were direct techniques for the estimation of the oxalate content in foods. CE can be used for foods with a medium (>10 mg/100 g) to high oxalate content due to a faster analysis time and lower running costs. IC may be better for the analysis of foods with a low oxalate content. HPLC-enzyme reactor method is low cost and very high sensitivity and selectivity (Honow R, Hesse A, 2002). Recently, Su J. et al (2010) developed a colorimetric method for determination of oxalate by using a copper ion and pyrocatechol violet (PV) which was a rapid observable visual color change from blue to yellow.

At present, Thai people often drink herbal teas and consumed local vegetables for their health benefits. However, some teas and vegetables may contain high oxalate content. Determination of the oxalate in these drinks and foods has advantage to consumer to prevent stone formation. A simple and rapid technique with low cost is required for oxalate determination in these samples.

Objective of the study

The aim of this study is to determine oxalate content in herbal teas and local vegetables by using a simple colorimetric method.

Methodology

Materials

Pyrocatechol violet (PV) and copper (II) nitrate were purchased from Fluka and Sigma, respectively. HEPES buffer (4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid) was purchased from Bio basic canada INC. The other chemicals used were of analytical grade.

Eighteen commercial herbal teas and 25 local vegetables were purchased from Khon Kaen market in October 2014.

Preparation and extraction of herbal teas

Herbal teas (1.5 g) were added with 30 ml of deionized water (DI) and heated at 93 ^{o}C for 5 minutes. The mixture was cooled to room temperature. Tea leaves residue was filtered through

Whatman No.4 filter paper and final volume was adjusted to 30 ml with DI according to a method of Pinitsoontorn C et al (2012).

Preparation and extraction of local vegetables

Fifteen gram of fresh vegetables was dried in a hot air oven at 50 °C for 24 hrs. The dried powder (1.5 g) was mixed with 30 ml of DI. The mixture was then heated at 93 °C for 5 minutes. After filtered with a filter paper, the final volume was adjusted to 30 ml with DI according to a method of Pinitsoontorn C et al (2012). This solution was used for oxalate determination.

Analysis of oxalate in teas and vegetables

To determinate oxalate content in teas and local vegetables by a method of Su J. et al (2010), 750 µl of 10 mM HEPES buffer pH6, 50 µl of 1 mM PV and 100 µl of 1 mM Cu (II) nitrate were mixed in a test tube. Then 100 µl of each sample was added into the solution. After standing for 1 minute, the absorbance at 443 nm was measured by using a spectrophotometer (Model Genesys 20, Thermo Scientific). Sodium oxalate at a concentration range 0-60 µg/ml was used for preparation of a standard curve. All experiment were done triplicate.

Results

Determination of oxalate in herbal teas and local vegetables

Oxalate contents in local vegetables and herbal teas are shown in Table 1 and Table 2, respectively. The local vegetables which contained the highest oxalate were *Careya sphaerica* Roxb. (0.0136 ± 0.0014 mg/g wet weight). The herbal teas named *Phyllanthus amarus* Schum & Thonn. had the highest oxalate content (0.0646 ± 0.0006 mg/g dry weight).

In this study, herbal teas had oxalate content range 0.0056-0.0646 mg/g dry weight and local vegetables had 0.0014-0.0136 mg/g wet weight. According to the Mann-Whitney test, the cutoff value was assigned as oxalate content 0.05 mg/g dry weigh, which concentration can obviously distinguish the difference between groups at p-values <0.05. About 5.55% of herbal teas had high oxalate whereas all the studied local vegetables are low oxalate content.

Discussion and Conclusion

The oxalate content in Thai herbal teas and local vegetables determined by colorimetric are lower than black tea (4.68 mg/g dry weight), green tea (0.23 mg/g dry weight) and U-long tea (1.15 mg/g dry weight) (Charrier MJS et al., 2002). However, Charrier MJS (2002) used HPLC technique to determine oxalate which is more accurate than colorimetric method.

The low oxalate content in the present study was supported by Po-ngern K (2003) that *Acacia pennata* (L.) Willd, *Coccinia grandis* (L.) Voigt, *Senna siamea* (Lam.) Irwin & Barneby had low oxalate crystals.

As Chanapa P (2011) recommended that consuming 22 g oxalic acid/60 kg body weight/day is toxic, consuming high dose oxalate vegetable and tea should be avoided because high oxalate foods leads to high risk of stone formation. In the present study, drinking herbal teas is probably safe because of their low oxalate compared to Chinese and Japanese teas.

The local fresh vegetables are also safe as side dishes when eat less than 1 kg per day.

In conclusion, the colorimetric method can be used for screening level of oxalate in teas and vegetables. The studied teas and vegetable are not high risk food for stone formation, but eating in a large amount should be considered.

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Table 1 Oxalate contents in Thai local vegetables used for this study with the information on their scientific name, Thai name and traditional uses.

Sample No.	Scientific name	Thai name	Traditional uses	Oxalate content	
				mg/ g wet weight	ppm
1	*Wolffia globosa* Roxb.	Kai-Num (Swamp Algae)	Source of high calcium[a]	0.0014 ± 0.0001	729.78
2	*Sesbania grandiflora* L. Desv.	Kae-Ban (Agasta)	Detoxification Fever treatment[b]	0.0017 ± 0.0001	715.47
3	*Psophocarpus tetragonolobus* Linn.	Tua-poo (Winged bean)	Reduce body heat[c]	0.0021 ± 0.0001	944.31
4	*Gynura procumbens* Lour. Merr.	Pae-Tum-Pueng	Reduce poisonous from insect bites[d]	0.0029 ± 0.0001	865.65
5	*Amaranthus lividus* Linn.	Pak-Hom (Amaranth)	Source of antioxidant[c]	0.0030 ± 0.0000	922.86
6	*Basella albe* Linn.	Plung (Ceylon Spinach)	Beta-carotene for maintain eyes[c]	0.0032 ± 0.0002	865.65
7	*Limnophila aromatica* Merr.	Pak-Ka-Yang	Fever treatment[c]	0.0032 ± 0.0001	922.86
8	*Coccinia grandis* L. Voigt	Tam-lung (Ivy Gourd)	Reduce flatulence[e]	0.0039 ± 0.0002	1087.33
9	Dolichandrone serrulata DC. Seem.	Kae-Pah	Antipyretics[c]	0.0039 ± 0.0003	944.31
10	*Garcinia cowa* Roxb.	Cha-Muang (Cowa)	Fever treatment[c]	0.0040 ± 0.0000	722.62
11	*Telosma minor* Craib	Dok-Ka-Jon (Cowslip creeper)	Periodic maintenance[c]	0.0042 ± 0.0000	887.10
12	*Senna siamea* Lam. Irwin & Barneby	Kee-lek	Diuretic[c]	0.0044 ± 0.0003	651.11

Table 1 Oxalate contents in Thai local vegetables used for this study with the information on their scientific name, Thai name and traditional uses. (Cont.)

13	*Centella asiatica* Linn. Urban.	Bua-Bong	Enhanced tropical urinary tract[c]	0.0046 ± 0.0000	1098.06
14	*Eryngium foetidum* L.	Pak-She-fa-Rang (Culantro)	High antioxidant[c]	0.0047 ± 0.0000	879.95
15	*Anethum graveolens* Linn.	Pak-Chee-Lao	High antioxidant[c]	0.0048 ± 0.0001	933.58
16	*Leucaena glauca* Benth.	Kra-Tin (Lead Tree)	Reduce blood sugar levels[c]	0.0048 ± 0.0003	608.21
17	*Neptunia oleracea* Lour.	Kra-Ched (Water mimosa)	Detoxification fever[f]	0.0049 ± 0.0000	869.22
18	*Piper sarmentosum* Roxb.	Cha-Plu	Colic treatment[c]	0.0052 ± 0.0001	790.56
19	*Caesalpinia mimosoides* Lamk.	Ka-ya	Blood maintain[g]	0.0057 ± 0.0002	604.00
20	*Passiflora foetida* L.	Ka-Tog-Rok (Fetid passionflower)	Reduce fever[c]	0.0065 ± 0.0001	1094.48
21	*Barringtonia acutangula* L.	Kradone-Num	Diarrhea treatment[h]	0.0068 ± 0.0003	1480.65
22	*Persicaria odorata* Lour.	Paew	Diuretic and flatulence[i]	0.0074 ± 0.0002	990.79
23	*Acacia pennata* L. Willd.ssp.	Cha-om	Reduce body heat[c]	0.0083 ± 0.0005	1098.06
24	*Azadirachta indica* A. Juss	Sa-dow	Periodic maintenance[b]	0.0107 ± 0.0002	1137.39
25	*Careya sphaerica* Roxb.	Kradone-bok	Healing wound[c]	0.0136 ± 0.0014	2049.16

[a] http://th.wikipedia.org (accessed date: 1 January 2015)

[b] http://www.rspg.or.th (accessed date: 1 January 2015)

[c] http://frynn.com (accessed date: 1 January 2015)

[d] http://www.pharmacy.mahidol.ac.th (accessed date: 1 January 2015)

[e] http://hq.prd.go.th (accessed date: 1 January 2015)

[f] http://xn--o3cepkej9b3gpeg.net (accessed date: 1 January 2015)

[g] http://library.cmu.ac.th (accessed date: 1 January 2015)

[h] http://www.likemax.com (accessed date: 1 January 2015)

[i] http://prayod.com (accessed date: 1 January 2015)

Table 2 Oxalate contents in Thai herbal teas used for this study with the information on their scientific name, Thai name and traditional uses.

Sample No.	Scientific name	Thai name	Traditional uses	Oxalate content	
				mg/ g dry weight	ppm
1	*Ganoderma lucidum* Reishi.	Hed-Lhinn-Jeu (Linzhi or reishi)	Anti-cancer[a]	0.0056 ± 0.0004	148.00
2	*Acanthus ebracteatus* Vahl.	Ngeug-Pla-Mor (Sea holly)	Treat skin diseases[b]	0.0123 ± 0.0004	328.00
3	*Derris scandens* Benth.	Kra-Sai-Toa-Wan-Prieng (Jewel vine)	Medicine as muscle pain relief, diuretic and emmenagogue[b]	0.0165 ± 0.0013	438.67
4	*Momordica charantia* Linn.	Ma-Ra_Khi_Nok (Bitter cucumber	Anti-diabetic[b]	0.0169 ± 0.0019	453.33
5	*Andrographis paniculata* Wall. Ex Nees.	Fah-Ta-Lai-Joan (Chiretta)	Antiinfections and antipyretics[b]	0.0191 ± 0.0015	508.00
6	*Aloe barbadensis* Mill.	Whan-Khang-Jor-Ra-Kae (Aloe vera)	Multipurpose skin treatment[b]	0.0198 ± 0.0010	528.00
7	*Murdannia loriformis* Hassk.	Yar Pak_Khing (Rolla Rao et Kammathy)	Antimutagenic activity and cancer chemopreventive activity[b]	0.0199 ± 0.0026	528.00
8	*Glycyrrhiza glabra* Linn.	Cha-Aim-Thed (Licorice)	Treatment of peptic ulcers, asthma, pharyngitis, malaria, abdominal pain, insomnia, and infections[b]	0.0206 ± 0.0004	548.00
9	*Thunbergia laurifolia* Linn.	Rhang-Jeud	Anti-inflammatory, antioxidant and hepatoprotective[b]	0.0214 ± 0.0005	568.00
10	*Orthosiphon grandiflorus* Bolding	Yar-Nuod_Maew (Cat's whisker)	Anti-allergic[c]	0.0223 ± 0.0002	593.33

Table 2 Oxalate contents in Thai herbal teas used for this study with the information on their scientific name, Thai name and traditional uses. (Cont.)

11	*Ginkgo biloba* L.	Pae-Gouy (Ginkgo)	Treatment blood disorders and Alzheimer's disease[b]	0.0225 ± 0.0008	598.67
12	*Phyllanthus emblica* Linn.	Ma-Kham-Pom (Indian gooseberry)	Treatment of diarrhea, jaundice and, inflammation, reduce phlegm [d]	0.0226 ± 0.0000	604.00
13	*Cassia angustifolia* Vahl.	Ma-Kham-Khak (Senna)	Reduce constipation[b]	0.0236 ± 0.0015	629.33
14	*Rhinacanthus nasutus* Kurz.	Thong-pan-Chung	Anticancer and antioxidant , anti-ulcer[c]	0.0272 ± 0.0003	724.00
15	*Senna alata* Linn.	Chum-Hed-Thed (Ringworm bush)	Reduce constipation[b]	0.0277 ± 0.0004	738.67
16	*Stevia rebaudiana* Bertoni	Yar-Wharn (Stevia)	Treatment of diabetes[b]	0.0311 ± 0.0009	829.33
17	*Pluchea indica* L. Less	Kluu (Indiun march fleabane)	Inhibitory activity against intestinal maltase[c]	0.0396 ± 0.0008	1054.67
18	*Phyllanthus amarus* Schum & Thonn.	Look-Tai-Bai	Anti-diabetic[e]	0.0646 ± 0.0006	1288.00

[a]http://health.kapook.com (accessed date: 1 January 2015)

[b]http://frynn.com (accessed date: 1 January 2015)

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