

1-1-2013

## QUANTITATIVE ANALYSIS OF EPIGALLOCATECHIN GALLATE IN YOUNG AND MATURE ASSAM TEA LEAF EXTRACTS

Pattra Ahmadi Pirshahid

Thongchai Hemthanon

Yaowaluk Khamphan

Phatsuda Chueboonmee

Chuleratana Banchonglikitkul

Follow this and additional works at: <https://digital.car.chula.ac.th/tjps>

 Part of the [Pharmacology Commons](#)

---

### Recommended Citation

Pirshahid, Pattra Ahmadi; Hemthanon, Thongchai; Khamphan, Yaowaluk; Chueboonmee, Phatsuda; and Banchonglikitkul, Chuleratana (2013) "QUANTITATIVE ANALYSIS OF EPIGALLOCATECHIN GALLATE IN YOUNG AND MATURE ASSAM TEA LEAF EXTRACTS," *The Thai Journal of Pharmaceutical Sciences*: Vol. 38: Iss. 0, Article 17.

Available at: <https://digital.car.chula.ac.th/tjps/vol38/iss0/17>

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Pharmaceutical Sciences by an authorized editor of Chula Digital Collections. For more information, please contact [ChulaDC@car.chula.ac.th](mailto:ChulaDC@car.chula.ac.th).

## QUANTITATIVE ANALYSIS OF EPIGALLOCATECHIN GALLATE IN YOUNG AND MATURE ASSAM TEA LEAF EXTRACTS

Pattra Ahmadi Pirshahid\*, Thongchai Hemthanon, Yaowaluk Khamphan, Phatsuda Chueboonmee and Chuleratana Banchonglikitkul

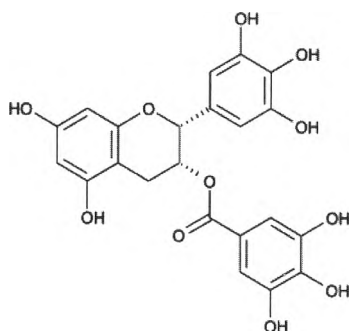
Pharmaceutical and Natural Products Department, Thailand Institute of Scientific and Technological Research, Technopolis, Moo 3, Klong 5, Klong Luang, Pathum Thani 12120, Thailand.

\*Corresponding author: [pattra\\_a@tistr.or.th](mailto:pattra_a@tistr.or.th) (Pattra Ahmadi Pirshahid)

**KEYWORDS:** Epigallocatechin gallate (EGCG), Assam tea

### INTRODUCTION

Assam Tea, *Camellia sinensis* var. *assamica* (Mast.) in Theaceae family, is widely distributed both in Asia and North America. Tea has been considered as a medicinal herb and a healthy beverage since ancient times. Nowadays, tea is one of the most consumed beverages in the world because of a major source of flavonoids and its anti-oxidant properties. Epigallocatechin gallate (EGCG) is a phenolic compound and a major component in tea leaves. EGCG have been reported to be an effective in health benefits such as against cardiovascular diseases, cerebral hemorrhage and lower risk of several types of cancer such as endometrial adenocarcinoma. Due to the health promoting of EGCG, TISTR has developed the dietary supplement products that contained the extract of young tea leaves which was available in market as green tea to be a part of active ingredient. The objective of this study is to analyse the quantity of EGCG in comparison between young and mature leaves in order to substitute the mature to the young leaves extract to lower cost of raw material by High performance liquid chromatography.



**Figure 1** Chemical structure of Epigallocatechin gallate (EGCG).

### MATERIALS AND METHODS

**Material** The leaves of Assam tea (*Camellia sinensis* Var. *assamica* (Mast.)) were collected in June 2013 from Wieng Pa Pao, Chiang Rai Province, Thailand. The first 5 leaves were collected as young leaves while the sixth up to the end of stem were collected as a mature leaves. Solvent used for extraction was 95% ethanol (commercial grade) which was obtained from The Liquor Distillery Organization. Solvents used for Liquid Chromatography were dimethylformamide, methanol, acetic acid and deionized water. All solvents were HPLC grade and obtained from RCI Labscan, Ireland. An authentic standard of Epigallocatechin gallate was HPLC grade with 95.0% purity from Sigma-Aldrich, USA.

**Plant material preparation** The young leaves (YL) and mature leaves (ML) were separately washed with water then dried at 45°C in oven for 20 hrs. The dried leaves were powdered for further extraction.

**Extraction** Ten gram of each young and mature powdered leaves of *Camellia sinensis* var. *assamica* (Mast.) were extract with 95% ethanol by stirring at room temperature for 20 min. The solid was separate from the mother liquor by filtration, followed by re-extraction for 4 times, the filtered solution was

combined, then evaporated under vacuum at 40°C to yield the crude extracts of young and mature leaves (YL,ML).

**Apparatus** HPLC analysis was performed on the Water 600 controller series connected with the Water 486 Tunable Absorbance Detector. Data analysis was performed by using Clarity chromatography software (DataApex, Czech Republic). Separation was comprised of a C<sub>18</sub> reverse phase column ODS HYPERSIL, 200x4.6 mm, 5 µm (Thermo Electron Corporation, USA). The mobile phase consisted of dimethylformamide/methanol/acetic acid and water (gradient), 1 ml/min as a flow rate with the injection volume of 20 µl and λ<sub>max</sub> is 278 nm.

**Preparation of standard solution** Epigallocatechin gallate (EGCG) was accurately weighed to 10 mg in a volumetric flask (50 ml) and the volume adjusted to 50 ml with water. The stock solution was diluted to create five concentrations for standard curve.

**Preparation of samples** One gram of each crude extract of YL and ML was dissolved with 50 ml of water. The solution was sonicated for 15 min, filtered, then adjusted to final volume of 50 ml, and filter through a 0.45 µm prior inject to HPLC.

**Calibration curve and linearity** The calibration curve was analysis of the standard epigallocatechin gallate at five concentrations. The linearity was determined by means of linear regression analysis. The calibration curve showed a coefficient of correlation  $R^2 = 0.9990$ .

**Limits of detection (LOD) and quantification (LOQ)** LOD and LOQ were the concentrations that give a signal to noise ratio equal to 3.35 and 11.17 respectively.



Figure 2 Young leaves (1) Mature leaves (2) Harvesting of tea leaves (3).

## RESULTS AND DISCUSSION

The extract of YL and ML were determination by HPLC as gradient elution with triplicate injects of solutions and gave good resolution without any interference by other compounds in the samples under the chromatographic condition. The retention time of EGCG was about 20.3 min. It revealed that the EGCG content in young leaves and mature leaves are  $0.73 \pm 0.05$  and  $2.86 \pm 0.02\%$ , respectively.

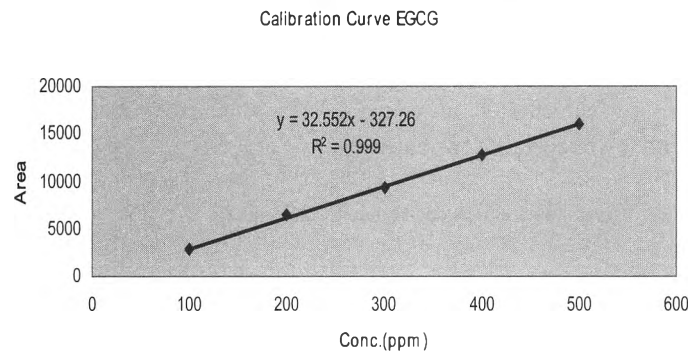


Figure 3 Calibration Curve of Epigallocatechin gallate (EGCG).

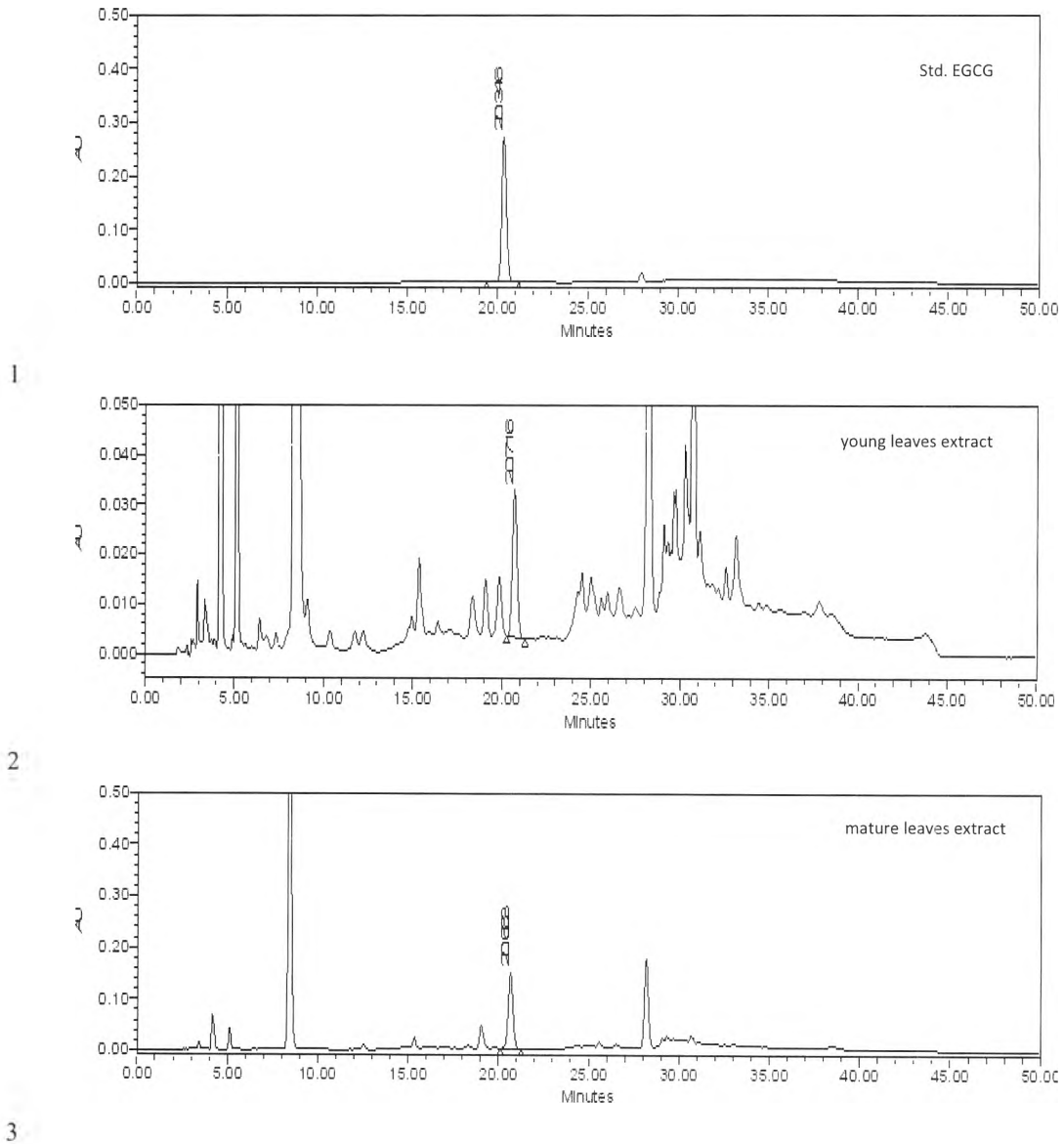


Figure 4 HPLC chromatogram of Standard Epigallocatechin gallate (EGCG)(1) young leaves extract (2) and mature leaves extract (3).

## CONCLUSION

The two different crude ethanolic extract of young and mature Assam tea leaves displayed a good percentage of EGCG content by  $0.73\pm 0.05$  and  $2.86\pm 0.02\%$  respectively. This result will be a value added to mature leaves by substitution to young leaves as an active ingredient of TISTR dietary supplement products with lower cost of raw materials.

**Table 1** Percentage of EGCG in Young leaves extract and Mature leaves extract.

Sample	EGCG (% w/w of the extract)
Young leaves extract (YL)	$0.73\pm 0.05$
Mature leaves extract (ML)	$2.86\pm 0.02$

## REFERENCES

1. <http://www.enzolifesciences.com/ALX-270-263/epigallocatechin-gallate/>
2. Anderson, R.F. et al. 2001. Green tea catechins partially protect DNA from OH radical induced strand breaks and base damage through fast chemical repair of DNA radicals. *Carcinogenesis*, v.22, n.8, p. 1.189-1.193.
3. Kawai, K. et al. 2003. Epigallocatechin gallate, the main component of tea polyphenol, binds to CD4 and interferase with gp120 binding. *J. Allergy Clin. Immunol.*, v. 112, n. 5, p. 951-7.
4. Rijken, P.J et al. 2000. Antioxidant and Other Properties of Green and Black Tea, in: Cadenas, E; Packer, L. *Handbook of Antioxidants*. 2<sup>st</sup> ed.: Marcel Dekker, New York, cap. 19, p. 371-399.
5. Sano, J. et al. 2004. Effects of Green Tea Intake on the Development of Coronary Artery Disease, *Circ J.*, v. 68, p. 665-70.
6. Sato, T.; MYATA, G. 2000. The Nutraceutical Benefit. Part I: Green tea. *Nutrition*, v. 16, p. 315-317.
7. Suzuki, M. et al. 2004. Protective effect for green tea catechins on cerebral ischemic damage. *Med. Sci. Monit.*, v. 10, n. 6, p. 166-74.
8. Drinking green tea modestly reduces breast cancer risk. *J Nutr.* 2009 Feb; 139(2): 310-6. Epub 2008 Dec 11.