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Effect of Boiling on Anhydrobarakol Content of Khilek (*Siamese cassia*) Young Flowers

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Abstract: Khilek, locally called of *Siamese cassia* is a medicinal plant of Thailand. Barakol, an active principle of Khilek, exhibits as anxiolytic properties, improving quality of sleep. Anhydrobarakol is a barakol that is lost a molecule of water. Khilek young flowers (KF) curry is an ancient curry of south of Thailand but they have bitter taste so they are boiled with water 1-2 times before they are used to curry. So this project was to study on the effects of boiling KF with water containing sodium chloride and turkey burry, the optimum time (min) of KF boiling for curry making and curry's nutrition. Anhydrobarakol content and sensory characteristics of boiled KF with water, boiled KF with water containing sodium chloride and boiled KF with water containing turkey burry were not significantly different ($p>0.05$). Anhydrobarakol content in boiled KF was decreased when increasing of time. KF for curry should be boiled one time in water 3 times of their weight, 5 min /time. KF curry had very low anhydrobarakol content (2.56 mg by fresh weight) and has much less tendency to cause liver toxicity.

Key words: Siamese cassia, young flowers, anhydrobarakol

INTRODUCTION

Siamese cassia, *Cassia siamea (Lamk.) Irwin et Barneby*, locally called "Khilek", a Thai medicinal plant belonging to the family *Caesalpiniaceae*. Many parts of it have been used for a long time as medicine: the roots as antipyretic and leaves for constipation, hypertension, insomnia and asthma (Ahn *et al*., 1978), the stem bark for against constipation, malaria and associated diseases such as fevers and jaundice (Ahn *et al*., 1978; Nsonde-Ntandou *et al*., 2005 and Kaur *et al*., 2006). The flowers are potent against insomnia and asthma; they are also commonly used in curries in India, Myanmar and Thailand. Their compounds includes barakol, emodin, beta- and gamma-sitosterol, lupeol, luteolin, d-pinitol, chromone alkaloids, flavonoid glycoside (Ahn *et al*., 1978; Shafiullah *et al*., 1995). An alcoholic extract of Siamese cassia flowers have high antioxidant activity and protect against oxidative damage in the liver (Kaur *et al*., 2006). Rattanapun *et al*. (2012) and Singkong *et al*. (2011) studied Khilek flower tea making. The main active constituent (barakol) in the flowers and young leaves of Siamese cassia was first extracted in 1969 (Hassanali-Walji *et al*., 1969) and identified as 3,4-dihydro-3,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene, also known as 2,5-dimethyl-3_H-pyrano-[2,3,4-de]-1-benzopyran-3a,8-diol and was produced using a synthetic procedure by Bycroft (Bycroft *et al*., 1970). Barakol occurs as pale lemon-yellow needle crystals, is unstable and converted to anhydrobarakol ($C_{13}H_{10}O_3$) by losing a molecule of water (Fig. 1) (Thongsaard, 1998). Barakol has been extracted for ongoing studies, including: its functions in antihypertensive activity (Ahn *et al*., 1978); as an anxiolytic agent in exploratory behavioral activities (Arunlakshana, 1949; Thongsaard *et al*., 1996); hypotensive activity (Suwan *et al*., 1992) and sedative activities (Bulyalert, 1993; Wongwitdecha *et al*., 2009). However, toxicity of barakol was also many reports as Jantarayota (1987) found that barakol produced acute toxicity and death in mice with LD50 = 324.09 mg/kg by intraperitoneal injection. Barakol may disrupt liver function and an increase of bilirubin, especially at the 240-mg/kg dose (Pumpaisalchai *et al*., 2005). Busarakumtragul *et al*. (2010) studied effects of barakol on vascular functions in rats and found that barakol reduced vasorelaxation uncompletely and presumably continuous consumption of barakol might not be suitable for health.

Barakol content in Khilek young Flowers (KF) was only studied by Padumanonda *et al*. (2007) with TLC-densitometric method. It found that KF contained 1.43% dry weight. KF curry is ancient and popular curry of Thailand especially people from south of Thailand. Due to KF is so bitterness from cassiamin, it is boiled 1-2 times and this process they usually add sodium chloride or turkey burry to reduce the bitterness that it is still not studied of their effect so this project was to study on the

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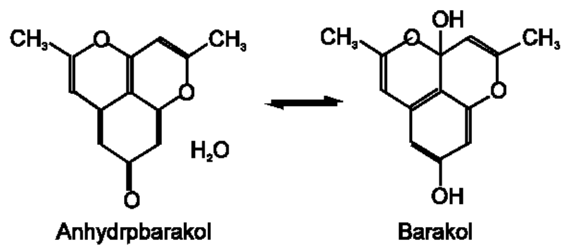

Fig. 1: Structure of barakol and anhydrobarakol (Thongsaard, 1998)

effect of boiling with water containing sodium chloride and turkey burry, the optimum time (min) of KF boiling for curry making and KF curry's nutrition especially its anhydrobarakol.

MATERIALS AND METHODS

Raw materials: Fresh KF were collected from Samutprakarn province of Thailand, packed in plastic bag and kept 1 night at 10°C. Sodium chloride and turkey burry (Solanum torvum Sw.) were purchased from Thai market. Anhydrobarakol standard was from the Government Pharmaceutical Organization, Thailand.

Effect of sodium chloride and turkey burry in KF boiling process: The CRD (Completely randomize design) experimental design, 3 replications, was conducted as three treatments of 1) KF boiling with only water (KFW), 3 times of KF weight 2) KF boiling with water containing sodium chloride 2 % of KF weight (KFS) and 3) KF boiling with water containing turkey burry (Solanum torvum) 10% of KF weight (KFT). All treatments were boiled with gas stove about 3 min. After boiling the boiled KF was blended and determine for color (a spectrophotometer (Spectraflash 600 plus, Data-color International, USA),CIE color values were recorded as L* = lightness (0 = black, 100 = white), a*(-a* = greenness, +a* = redness), b*(-b* = blueness, +b* = yellowness), c* (chroma) h*(hue angle = arctangent b/a), sensory evaluation of KF curry from south of Thailand recipe using 30 panels and 7-point hedonic scale (1 = dislike very much and 7 = like very much) for color, aroma, flavor, texture and overall. The boiled filtrates were determine for Total Soluble Solid (TSS) using hand refractometer, Atago, pH using pH meter, Orion, Model 410. The data was analyzed by using Analysis of Variance and Duncan New's Mutiple Range Test for mean comparison at 0.05 significant level by statistical software SPSS (version 12).

Anhydrobarakol determination: The anhydrobarakol content was determined by High Performance Liquid Chromatography (HPLC, Agilent 1100 Series). The sample (1-2 g) was added methanol (mL), 2 times of its weight, followed by shaking with vortex mixer for 1 min and extracted with ultrasonic (CREST) at 30°C for 15 min after that it was shaken again with vortex mixer for 1 min. Subsequently, the mixture was centrifuged (Mini-centrifuge, model No. C-1200) for 2 min and the supernatant was filtered with membrane filter (ϕ 0.45 µm). The extraction (1 µL) of sample was separated with mobile phase and analyzed by HPLC method using Bondapak column C18 125°A, 39*300 mm with the following parameters: diode array detector, Acetronitrile: MeOH (80:20) mobile phase, flow rate 1 mL/min, using a UV spectrophotometer detector at 241 and 366 nm. The result of peak area was compared with the anhydrobarakol standard in each wavelength using the linear equation based on the calibration curve. Extraction and clean-up procedure were optimized with confirmed positive and spiked samples. This analyst was used one QC check standard and one duplicate sample analysis pair for validation. Equation 1 was used to determine the anhydrobarakol content (dry weight).

$$\text{Anhydrobarakol, mg/1000g.} = C * V1*1000 / Wt * V2 \quad (1)$$

Where:

C = Anhydrobarakol content (mg)
V1 = Sample volume (mL)
Wt = Sample weight (g)
V2 = Injection volume (mL) to HPLC (mL)

Effect of times (min) of KF boiling: The CRD, 3 replications, was conducted as 3 treatments as KF boiling at 3, 5 and 7 min. All treatments were boiled with gas stove only one time. After boiling the boiled KF was kept at -13°C to -15°C. After that the boiled KF was thawed for sensory evaluation of KF curry from south of Thailand recipe using by 7-point hedonic scale and some parts of them were blended to determine color and anhydrobarakol content. The boiled filtrates were determine for color, TSS, pH. The data was analyzed as the same upper part.

The nutrition of KF curry: The KF curry was analyzed in triplicate for chemical quality using moisture (T-CM-002 Based on AOAC (2000) 925.45, fat (T-CM-075 Based on AOAC (2000) 989.05, protein (T-CM-003 Kjeldahl Method: Based on AOAC (2000) 991.20, using 6.25 as the conversion factor), crude fiber (T-CM-077 Based on AOAC (2000) 978.10, ash (T-CM-001 Based on AOAC (2000) 938.08 and carbohydrate contents (using calculations by 100- %moisture-% fat-% protein-%ash-% crude fiber), calcium (T-CM-020 Based on AOAC (2000) 984.27, phosphorus and iron (T-CM-020 Based on AOAC (2000) 999.10. Anhydrobarakol content of curry was calculated from boiled KF.

KF curry recipe of south of Thailand, 1000 g:

- (a) The fresh KF, 186 g were boiled with water 3 times of theirs weight, 5 min after that they were filter with colander
- (b) The ingredients of curry as 8 g of dried small red chili, 6 g fresh small red chili, 4 g of turmeric, 14 g of lemon grass, 10 g of garlic, 4 g of galangal, 2 g of sodium chloride, 2 g black pepper and 10 g of shrimp paste sauce were blended
- © Coconut milk, 480 g was added into the pot and heated until it was boiled after that the ingredients from (b) were added and heated until they were boiled then boiled KF from (a) were added and 36 g of roasted fish were added then the curry was boiled 10 min
- (d) The curry was flavored with 30 g of fished sauce, 6 g of palm sugar, 2.5 g of bergamot leaves and 0.2 g monosodium glutamate after that it was heated to boil

RESULTS AND DISCUSSION

Effect of sodium chloride and turkey burry in KF boiling process: Due to before KF curry making, Thai people must boil KF with water and usually add sodium chloride or turkey burry into the water for improving the reduction of bitterness. It found that the filtrate (Fig. 2) was significantly different in TSS. The filtrate of KFS was the highest TSS while the others treatments were not significantly different because salt is a water-holding capacity of foods (Gibson *et al.*, 2000) so it provided TSS. The pH was showed mild acidity about 4.90-5.01 that cause to the sour taste. For the color of the filtrate (Fig. 3), it found that only L*, a* and h* was significantly different. The L* of the filtrate from KFS was highest value while the others were not significantly different because sodium chloride was dissolved into the filtrate resulting to the less lightness. The a* of the filtrate from KFT was lowest value while the others was not significantly different because the green color from turkey burry may be dissolved into the filtrate resulting to more greenness color. The h* that was shown in general color of yellow color, was found that the filtrate of 3 treatment was in quadrant 2 and closed to 90 degree and the filtrate of KFT was the highest value so its color was more yellow color than the others however, overall color of the filtrate from 3 treatments provided yellow mixed mild green color.

For the color of the boiled KF (Fig. 4), it found that only L* had significantly different. The L* of boiled KFT was highest value that according to its filtrate from Fig. 3. However, overall color of the boiled KF from 3 treatments provided yellow mixed mild red color.

The anhydrobarakol (The linearity for the response of anhydrobarakol was assessed of 0.2, 0.4 and 0.6 calibration lines were represented by linear equation: y

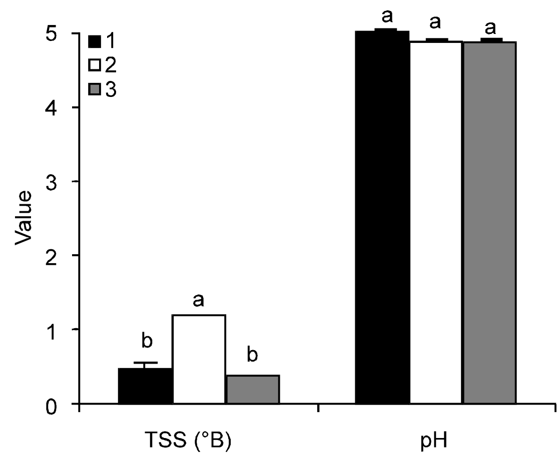

Fig. 2: TSS and pH of the filtrate, 1= KFW 2= KFS 3 = KFT, Mean with the same letters were not significantly different ($p > 0.05$), (T indicates the upper range of the mean plus the standard deviation)

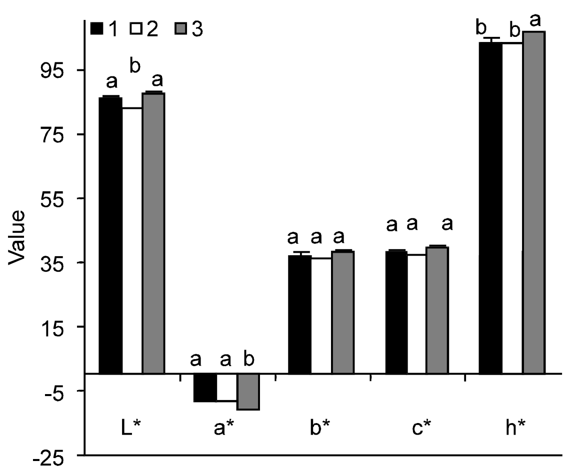

Fig. 3: Color (L* a* b* c*and h*) of the filtrate, 1= KFW 2 = KFS 3 = KFT, Mean with the same letters were not significantly different ($p > 0.05$), (T indicates the upper range of the mean plus the standard deviation.)

= 592.97x+3.1111 (r^2 = 0.9989). Accuracy was determined equation by percentage recovery. Anhydrobarakol from three treatments (Fig. 5) was not significantly different as 132.32-138.53 mg/kg or 0.0132-0.0138 % by fresh weight.

The sensory test of KF curry was evaluated by male 4 people and female 26 people; 24-57 years old (Fig. 6) replacing boiled KF only because most people consume KF in curry. It showed that every attributes were not significantly different among treatments although, sodium chloride is an effective bitter suppressant (Keast *et al.*, 2004; Breslin and Beauchamp,1995) and high

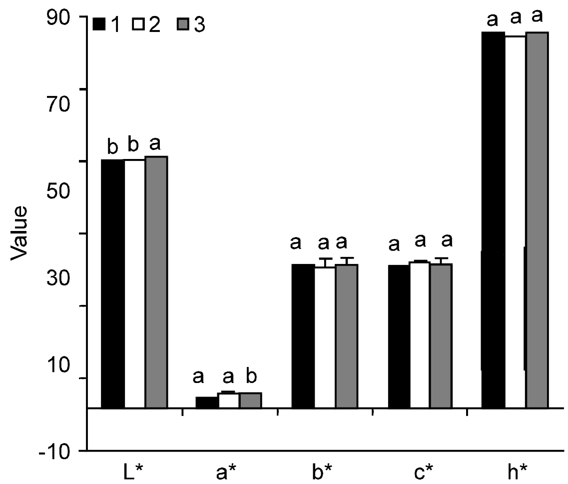

Fig. 4: A color (L* a* b* c* h*) of boiled KF, 1= KFW 2= KFS 3 = KFT, Mean with the same letters were not significantly different (p > 0.05), (T indicates the upper range of the mean plus the standard deviation)

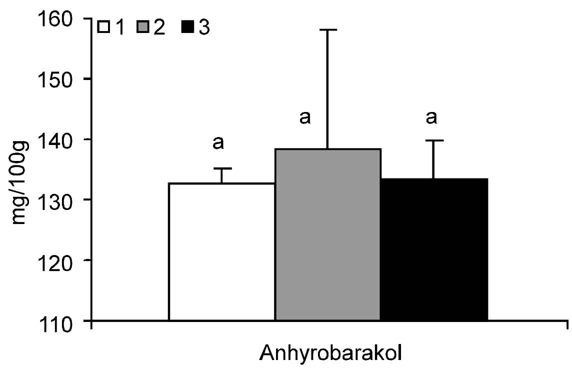

Fig. 5: Anhydrobarakol of the boiled KF curry, 1= KFW 2= KFS 3 = KFT, Mean with the same letters were not significantly different (p > 0.05), (T indicates the upper range of the mean plus the standard deviation)

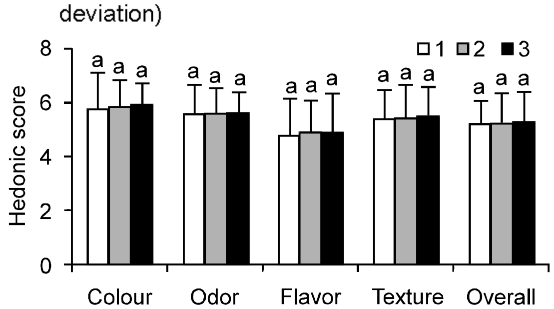

Fig. 6: Sensory evaluation quality of boiled KF, 1= KFW 2 = KFS 3 = KFT, Mean with the same letters were not significantly different (p > 0.05), (T indicates the upper range of the mean plus the standard deviation)

pectin of turkey burry may absorb the bitter substance because the filtrate was the most brightness. However, the concentrations of salt and turkey burry should study in the next time. However, due to high concentration of coconut milk and spices in curry so theirs flavor; especially the bitterness may be concealed. For the clear result of the sensory test, the only boiled KF should be evaluated. The bitter substance in KF may be soluble in water because the filtrate of all treatments was bitter taste by all authors (5 people); resulting to reduce the bitterness in boiled KF. All attributes obtained low average scores as little to moderate like that may be the bitterness in boiled KF was residue due to only 3 min in boiling.

Effect of time (min) of KF boiling: From the past data, especially anhydrobarakol and sensory test, it found that boiling with water, boiling with water containing sodium chloride and boiling with water containing turkey burry were not significantly different on quality of boiled KF so to save cost, we selected to boil KF with only water but it should be studied the optimum time (min) in boiling.

For the filtrate at Fig. 7, it found that only TSS was significantly different. The TSS of boiling at 3 min was lowest value while the others were not significantly different because 3 min of boiling was the less time than the others to extraction soluble solid from KF to the filtrate. For pH, it was not significantly different in 3 treatments and obtained the mild acid filtrate. For color of filtrate at Fig. 8 it found that L* a* b* c* and h* were not significantly different and overall color was yellow.

The anhydrobarakol of boiled KF (Fig. 9) was significantly different that boiled KF at 3 min obtained highest value but it was not significantly different with boiled KF at 5 min but it was significantly different with boiled KF 7 min. In generally the increased boiling time resulted the decreased quantity of anhydrobarakol in boiled KF. Due to anhydrobarakol may be soluble in water as the same barakol (Chaichantipyuth, 1979).

For the color of the boiled KF (Fig. 10), it found that only L* had significantly different. The L* of 7 min boiled young flowers was highest value. However, overall color of the boiled young flowers from 3 treatments provided yellow color.

The sensory evaluation of KF curry (male 4 people and female 26 people), 24-57 years old (Fig. 11) showed that most attributes were not significantly different among treatments except the flavors of 5 min boiled KF obtained highest value about moderately like but it was not significantly different from 7 min boiled KF So the save cost and 5 min boiled KF had anhydrobarakol content residue more than 7 min boiled KF so the optimum time to boiled KF was 5 min.

The nutrition of KF curry: By average, 100 g KF curry, fresh weight had moisture, fat, protein, ash, crude fiber,

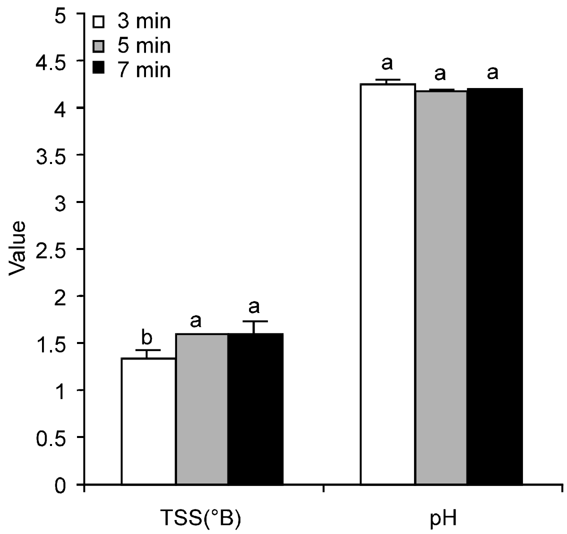

Fig. 7: TSS (°B) pH of the filtrate from boiled KF, Mean with the same letters were not significantly different ($p > 0.05$). (T indicates the upper range of the mean plus the standard deviation)

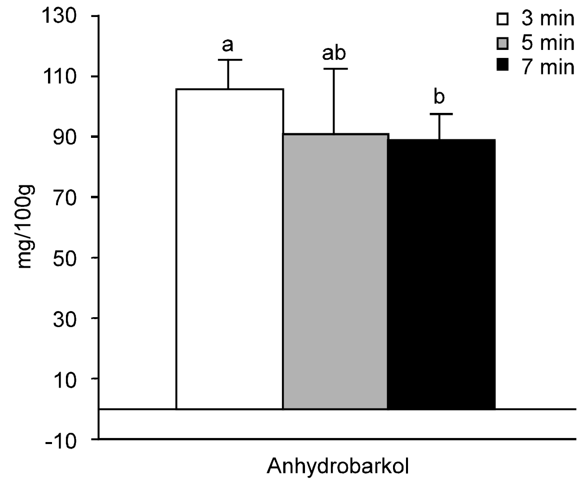

Fig. 9: Anhydrobarakol of boiled KF, Mean with the same letters were not significantly different ($p > 0.05$). (- indicates the upper range of the mean plus the standard deviation)

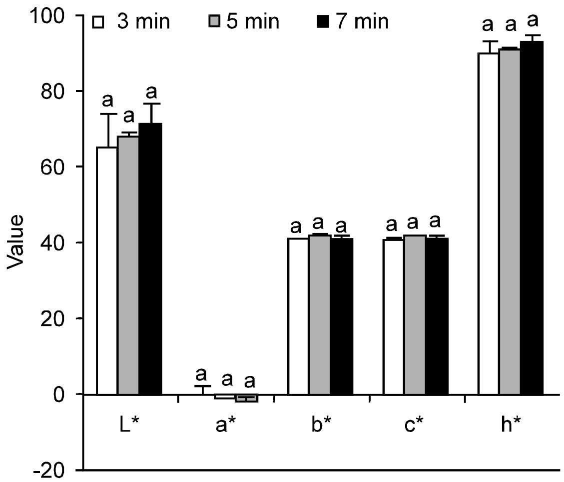

Fig. 8: Color (L* a* b *c* h*) of the filtrate from boiled KF, Mean with the same letters were not significantly different ($p > 0.05$). (T indicates the upper range of the mean plus the standard deviation)

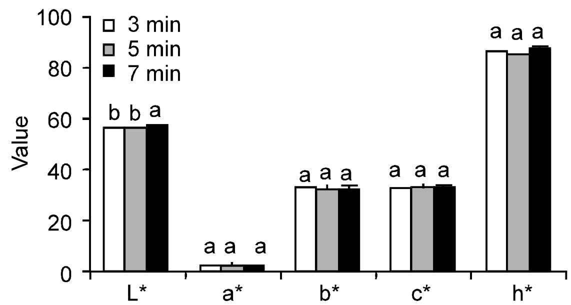

Fig. 10: Color (L* a* b* c* h*) of boiled KF, Mean with the same letters were not significantly different ($p > 0.05$). (T indicates the upper range of the mean plus the standard deviation)

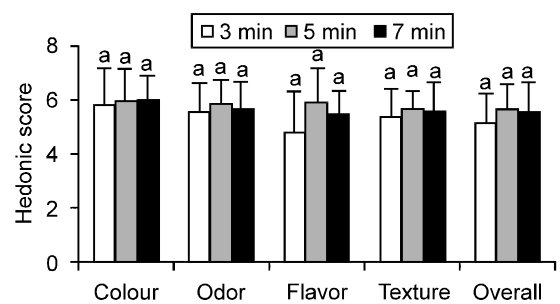

Fig. 11: Sensory evaluation quality of curry from 3, 5 and 7 min boiled KF, Mean with the same letters were not significantly different ($p > 0.05$). (T indicates the upper range of the mean plus the standard deviation)

carbohydrate, calcium, phosphorus and iron content about 77.99 g, 7.69 g, 4.67 g, 1.03 g, 4.13 g, 4.49 g, 42.22 mg, 71.08 mg and 0.63 mg, respectively. This menu had energy 4.43×10^5 J. It had 2.57 mg of the anhydrobarakol by calculation because the KF curry had high coconut milk and spices that was difficult to filtrate for analysis. In calculation, starting from 100 g KF curry used 18.60 g fresh KF which was 28.45 g boiled KF that contained 2.57 mg anhydrobarakol content. Anhydrobarakol content in the KF curry was very low and Thai people do not consumed it every day. Compared to the recommended dose of 2-4 Khilek capsules containing = 10 mg of anhydrobarakol (prodrug of barakol) per capsule (Hongsirinirachorn *et al*., 2003) so we can consumed 0.78-1.56 kg /day that is too much so barakol content from the consumption KF curry is unlikely to cause toxicity.

Conclusion: Anhydrobarakol content in boiled KF was decreased when increasing of time. KF for curry should be boiled one time in water 3 times of their weight, 5 min /time. KF curry had very low anhydrobarakol content (2.56 mg by fresh weight) and has much less tendency to cause liver toxicity.

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REFERENCES

AOAC, 2000. Official Methods of Analysis of AOAC International. 17th Edn, W. Horwitz, (Ed.). Maryland, USA.

Ahn, B.Z., U. Degen, C. Lienjayetz, P. Pachaly and F. Zymalkowski, 1978. Constituents of *Cassia siamea*. Arch Pharm (Weinheim), 311: 569-578.

Arunlakshana, O., 1949. Studies of indigenous drugs: 1. Pharmacological actions of the leaves of *Cassia siamea*. Siriraj Hospital Gazette, 1: 434-444.

Breslin, P.A. and G.K. Beauchamp, 1995. Suppression of bitterness by sodium: variation among bitter taste stimuli. Chem. Senses, 20: 609-623.

Bulyalert, D., 1993. Effects of barakol on the central nervous system: Quantitative analysis of EEG in the rat. Chiang Mai. Med. J., 32: 191-196.

Busarakumtragul, P., P. Tep-areenan, S. Chainakul and O. Wongsawatkul, 2010. Effects of barakol on vascular functions in rats. Int. J. Pharmacol., 6: 257-263.

Bycroft, B.W., A. Hassanali-Walji, A.W. Johnson and T.J. King, 1970. The structure and synthesis of barakol: A novel dioxaphenalene derivative from *Cassia siamea*. J.Chem. Soc., 12: 1686-1689.

Chaichantipyuth, C., 1979. A phytochemical study of the leaves of *Cussiasiamea* and *Cassiaspectabilis*. MS Thesis, Chulalongkorn University, Bangkok, Thailand.

Gibson, J., G. Armstrong and H. McIlveen, 2000. A Case for Reducing Salt in Processed Foods. Nutr. Food Sci., 30: 167-173.

Hassanali-Walji, A., T.J. King and S.C. Wallwork, 1969. Barakol, a novel dioxaphenalene derivative from *Cassiu siumeu*. J. Chem. Soc., Chem. Commun., 12: 678.

Hongsirinirachorn, M., S. Threeprasertsuk and A. Chutaputti, 2003. Acute hepatitis Associated with Barakol. J. Med. Assoc. Thai., 86: S484-489.

Jantarayota, P., 1987. Effects of barakol extracted from leaves of *Cassia siamea* on the central nervous system. Chulalongkorn University, Bangkok, Thailand. MS Thesis.

Kaur, G., M. Alam, Z. Jabbar, K. Javed and M. Athar, 2006. Evaluation of antioxidant activity of *Cassia siamea* flowers. J. Ethnophar. Macol., 108: 340-348.

Keast, R. S. J., T. M. Canty and P.A. S. Breslin, 2004. The Influence of Sodium Salts onBinary Mixtures of Bitter-tasting Compounds. Chem. Senses, 29: 431-439.

Nsonde-Ntandou, G.F., M. Ndounga, J.M. Ouamba, M. Gbeasso, A. Etou Ossebi, F. Ntoumi and A.A. Abena, 2005. Enquete ethnobotanique: Screening chimique et efficacite therapeutique de quelques plantes utilisees contre le paludisme en medecine traditionnelle a Brazzaville. Phytotherapie, 1: 13-18.

Padumanonda, T., L. Suntornsuk and W. Gritsanapan, 2007. Quantitative analysis of barakol content in *Senna siamea* leaves and flowers by TLC-Densitometry. Med. Princ Pract., 16: 47-52.

Pumpaisalchai, W., S. Kaewichit, W. Niwatananun and K. Sanichwankul, 2005. HPLC analysis method and pharmacokinetics of barakol. CMU. J., 4: 49-58.

Rattanapun, B., W. Singkong A. Halee and S. Thiangsanthia, 2012. Effects of Blanching Khi Lek (*Cassia siamea* Lamk) Flowers in Brine on Qualities of Khi Lek Flower Tea. RMUTP Res. J., 6: 81-87.

Shafiullah, M., M. Parveen, M. Kamil and M. Illyas, 1995. A new isoflavone C-glycoside from Cassia siamea. Fitoterapia, 65: 339-341.

Singkong, W., B. Rattanapun, A. Halee and S. Thiangsanthia, 2011. Product Development of Khi Lek Flower with Kek Hauy Flower Ready-to-drink Tea. J. Applied Sci., 10: 12-22.

Suwan, G., R. Sudsuang, D. Ghumma-Upakorn and C. Werawona, 1992. Hypertensive effects of barakol extracted from leaves of *Cassia siamea* Lam. in rats and cats. Thai J. Physiol. Sci., 5: 53-65.

Thongsaard, W., 1998. Physiological and pharmacological properties of *Cassia siamea* and its active constituent, barakol. Thai J. Physio Sci., 11: 1-26.

Thongsaard, W., C. Deachapunya, S. Pongsakorn, E.A. Boyd, G.W. Bennett and C.A. Marden, 1996. Barakol: A potential anxiolytic extracted from *Cassia siamea*. Pharmacol. Biochem. Behav., 53: 753-758.

Wongwitdecha, N., S. Srisomboonlert and P. Ritilert, 2009. Effect of barakol on the elevated plus-maze behavior of social and isolation reared rat. Proceedings of the 9th World Congress of Biological Psychiatry, Paris, France, June 28-July 2, 2009, pp: 34-34.