ORIGINAL ARTICLE

Pharmacognostic evaluation with reference to catechin content and antioxidant activities of pale catechu in Thailand

Thidarat Duangyod[1], Chanida Palanuvej[1], Nijsiri Ruangrungsi[1,2]

[1]College of Public Health Sciences, Chulalongkorn University, Bangkok, [2]Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathum Thani, Thailand

J. Adv. Pharm. Technol. Res.

ABSTRACT

Pale catechu, a well-known crude drug, has been widely used for anti-diarrhea. Due to its medicinal usage, this study was performed to evaluate the pharmacognostic and antioxidant properties as well as catechins contents of pale catechu in Thailand. Twenty samples of pale catechu collected from traditional drug stores throughout Thailand were investigated. Antioxidant activities, total phenolic, nontannin phenolic, and total tannin contents were evaluated. (+)-catechin and (−)-epicatechin were quantitatively analyzed by high performance liquid chromatography. The results revealed that most of pale catechu samples were adulterated according to high ash values. Qualified pale catechu in Thailand were demonstrated for their average contents of total ash, acid insoluble ash, loss on drying, and moisture as 5.20 ± 0.19, 1.61 ± 0.17, 13.14 ± 0.10, and 13.20 ± 1.07 g/100 g of dry weight, respectively. The ethanol and water soluble extractive matters were 91.66 ± 5.16 and 44.59 ± 3.18 g/100 g of dry weight respectively. (+)-catechin in theses samples was 478.87 ± 2.77 µg/mg of crude drug, whereas (−)-epicatechin was found to be trace (<limit of quantitation). The promising antioxidant activities were demonstrated compared to (+)-catechin hydrate.

Key words: (+)-catechin, (−)-epicatechin, antioxidant activity, pale catechu, quality evaluation

INTRODUCTION

Pale catechu or gambir is a water extract prepared from leaves and stems of *Uncaria gambir* (Hunter) Roxb. which belongs to Rubiaceae family. It is generally a small cylinder of reddish-brown color, light, and friable. Pale catechu has been used to treat diarrhea in Thai traditional medicine. The extract of *U. gambir* contains catechin, epicatechin, gambiriin A1, A2, B2, and B2.[1,2] Moreover, this extract showed high antioxidant activities.[3-6] According to previous reports, this crude drug is often found susceptible to adulteration.[7,8] The adulterations of herbal preparation are not easily distinguished from the right material using naked eyes. The standardization is an essential measurement for quality, purity, and adulteration of plant drugs.[9] The screening of bioactive compounds from the herbal extract is also important to new drug development.[10] Hence, to control the quality of raw medicinal materials, establishment of standardization parameter is needed. This research was attempted to evaluate the pharmacognostic parameters of pale catechu in Thailand, to investigate antioxidant activities, total phenolic, and total tannin contents, as well as to determine (+)-catechin and (−)-epicatechin

Address for correspondence:
Dr. Chanida Palanuvej,
College of Public Health Sciences, Chulalongkorn University,
Bangkok 10330, Thailand.
E-mail: chanida.p@chula.ac.th

Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/2231-4040.161505

How to cite this article: Duangyod T, Palanuvej C, Ruangrungsi N. Pharmacognostic evaluation with reference to catechin content and antioxidant activities of pale catechu in Thailand. J Adv Pharm Technol Res 2015;6:97-102.

contents in this crude drug by high performance liquid chromatograph (HPLC).

MATERIALS AND METHODS

Sample collection and extraction

Twenty samples of pale catechu were purchased from different Thai traditional drug stores in 18 provinces located at four regions of Thailand. Associate Professor Dr. Nijsiri Ruangrungsi authenticated all sets of crude drug. One milligram of each sample was mixed with 1 mL of ultra-pure water. Then, the mixture was filtered and diluted to evaluate the antioxidant activities, total phenolic, total tannin contents at the concentration of 100 µg/mL. For HPLC analysis, the concentration of 1 mg/mL was used, and the sample was filtered through a 0.45 µm PTFE membrane syringe filter (ANPEL Scientific Instrument, China) before chromatographic analysis.

Chemicals

(+)-catechin hydrate (CAS no. 225937-10-0, purity ≥98%), (+)-catechin (CAS no. 154-23-4, purity ≥99%), (−)-epicatechin (CAS no. 490-46-0, purity ≥98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and acetonitrile were obtained from RCI Labscan, Thailand. Formic acid was purchased from Fisher Scientific (Leicestershire, UK). Water used in this study was ultra-pure water prepared by SNW ultra-pure water system (NW20VF, Heal Force).

Instrumentations

An ultraviolet (UV)-spectrophotometer (UV-1800 model, Shimadzu, Japan) and a microplate reader (BiochromAsys UVM 340, Thailand) were used in this study. HPLC (Shimadzu DGU-20A3, Shimadzu, Japan) equipped with photodiode array detector (Shimadzu SPD-M20A, Shimadzu, Japan) was used for catechins analysis.

Macroscopic evaluation

Pale catechu was identified by visual examination of the physical properties such as size, color, texture, and other visual inspection. Whole plants of *U. gambir* was illustrated by hand drawing in proportional scale related to the real size.

Physico-chemical evaluation

Total ash, acid insoluble ash, loss on drying, moisture content, and extractive matters parameters of pale catechu were performed according to WHO guideline for quality control methods for medicinal plant materials as briefly described below:[11]

Three grams of ground sample was dried at 105°C to constant weight to determine loss on drying. Then, 3 g of ground sample was incinerated at 500°C until white to obtain the carbonless total ash. The ash was boiled with 25 mL of HCl (70 g/L); the insoluble matter was incinerated again at 500°C for 5 h to obtain the percentage of acid insoluble ash. Moisture content was conducted by azeotropic distillation method using water-saturated toluene. Determinations of extractive matters were carried out with 95% ethanol and water as solvents. Five grams of ground sample was macerated with 70 mL of the solvent under shaking for 6 h and standing for 18 h before filtration. The extract was filtered through Whatman No. 4 and adjusted to 100 mL after washing the marc. Twenty milliliters of the filtrate was evaporated on a water bath and further dried at 105°C until a constant weight was obtained.

Thin-layer chromatographic identification

The crude drug of pale catechu (1 g) was macerated with 95% ethanol for 6 h and then evaporated to dryness. The residue was dissolved in 1 mL of ethanol. Three microliters of the sample solution were applied onto a thin-layer chromatographic (TLC) plate coated with silica gel GF_{254}. The TLC plate was then placed in a chamber with chloroform, ethyl-acetate and formic acid (3:6:1, v/v/v) as mobile phase. After development, the plate was removed and allowed to dry at room temperature and examined under UV light with 254 nm and 365 nm. Then, the plate was sprayed with vanillin reagent and heated in an oven at 105°C for 5 min.

Antioxidant activities

2, 2-diphenyl-1-picrylhydrazyl assay

Five hundred microliters of sample (100 µg/mL) were mixed with 500 µl of 0.12 mol/m^3 2,2-diphenyl-1-picrylhydrazyl solution in methanol. The mixtures were kept in the dark for 30 min and optical density was measured at 517 nm. (+)-catechin hydrate was used as a positive control. Triplicate measurements were carried out. Percentage of scavenging activity was calculated by the formula given below:

$$\text{Scavenging activity (\%)} = ([\text{absorbance}_{control} - \text{absorbance}_{sample}]/\text{absorbance}_{control}) \times 100.$$

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) reagent was prepared according to the method of Benzie and Strain.[12] Briefly, the FRAP reagent was prepared by mixing 100 mL of 300 mol/m^3 acetate buffer pH 3.6 with 10 mL of 10 mol/m^3 2,4,6-tris (2-pyridyl)-s-triazine dissolved in 40 mol/m^3 HCl and 10 mL of 20 mol/m^3 $FeCl_3.6H_2O$ dissolved in ultra-pure water. Freshly prepared reagent was warmed at 37°C before used. One hundred microliters of each sample (100 µg/mL) were mixed with 700 µl of the FRAP reagent for 30 min under the dark conditions. The absorbance was measured at 593 nm. Aqueous solutions of $FeSO_4$ in the range of 0.1–1.0 mol/m^3 were used for the calibration curve. Results were expressed in mol/m^3 Fe (II)/mg of dry sample. (+)-catechin hydrate was also tested under the same conditions as standard antioxidant compounds. All samples were performed in triplicate.

Metal iron chelating assay

The chelating activity of the sample on Fe^{2+} was measured according to the method of Gupta *et al*.[13] One hundred microliters of sample (100 µg/mL) was incubated with 7.5 µl of 2 mol/m^3 $FeCl_2$ for 5 min. The reaction was started by addition of 30 µl ferrozine (5 mol/m^3). After 10 min, the absorbance of ferrous iron-ferrozine complex at 562 nm was measured using a microplate reader. Ethylenediamine triacetic acid (EDTA) served as positive control. All determinations were performed in triplicate. The ability of the sample to chelate ferrous ion was calculated using the formula given below:

$$\text{Chelating activity (\%)} = ([\text{absorbance}_{control} - \text{absorbance}_{sample}]/\text{absorbance}_{control}) \times 100.$$

Beta-carotene bleaching assay

Briefly, 1 mg of beta-carotene, 40 mg of linoleic acid, and 400 mg of Tween 20 were mixed in 4 mL of chloroform. Then, chloroform was removed at 40°C under vacuum. The mixture was immediately diluted with 100 mL of water then the mixture was vigorous agitated for 5 min using the ultrasonic bath to form an emulsion. Aliquots of the emulsion (1 mL) were transferred into cuvettes that contained 250 µl of the sample (100 µg/mL). The mixture was then gently mixed and placed in a water bath at 50°C for 180 min. The absorbance of the sample was recorded at 0 min and 180 min at 470 nm. All determinations were performed in triplicate. (+)-catechin hydrate was used as positive controls. The negative control was ultra-pure water. The degradation bleaching rates of beta-carotene was evaluated as the percent of antioxidant capacity using the following equation:

$$\text{Antioxidant capacity (\%)} = (1-[A_0 - A_{180}]/[C_0 - C_{180}]) \times 100.$$

A_0, A_{180}: Absorbance at zero time and end time of incubation for test sample respectively.

C_0, C_{180}: Absorbance at zero time and end time of incubation for test control respectively.

Total phenolic content

The total phenolic content of the sample was determined using the Folin–Ciocalteu reagent. Eight hundreds microliters of sample extracts (100 µg/mL) and 200 µl of 15% Folin–Ciocalteu reagent were added in the test tube then adjusted the volume to 2.0 mL with water. The mixture was left for 5 min. After that 1.0 mL Na_2CO_3 (0.106 g/mL) is added. The mixture was kept in the dark at room temperature for 60 min. The absorbance was measured at 756 nm. The results were expressed as micrograms of catechin equivalents per 100 µg dry weights of crude drug.

Total tannin content and nontannin phenolic content

Briefly, 3.5 mg of hide powder was weighed, and then 500 mL of sample (100 µg/mL) was added in the test tube. The mixture was shaken for 60 min afterwards centrifuged for 10 min and finally the supernatant was collected. The supernatant has only simple phenolic compounds other than tannins. The tannins would have been precipitated along with the hide powder. The phenolic content of the supernatant was then measured following the same procedure describe above. The content of nontannin phenols was expressed as micrograms of catechin equivalents per 100 µg dry weights of crude drug. Total tannin content was determined by subtraction of nontannin phenolic content from total phenolic content. All samples were performed in triplicate.

High-performance liquid chromatograph analysis

Preparation of standard solution

The stock solution of (+)-catechin and (−)-epicatechin were prepared by dissolving 1 mg of each compound in 1 mL of methanol. The solution was filtered through a 0.45 µm PTFE membrane syringe filter.

Chromatographic conditions

Shimadzu DGU-20A3 HPLC consisted of a binary solvent delivery system, an auto-sampler, a column temperature controller, and a photodiode array detector. System control and data analysis were processed with Shimadzu LC Solution software. The chromatographic separation was accomplished with an Inertsil ODS-3 column (5 µm × 4.6 mm × 250 mm) and an Inertsil ODS-3 HPLC Guard Column (5 µm × 4.0 mm × 10 mm) using water containing 0.1% formic acid (a) and acetonitrile containing 0.1% formic acid (b) as mobile phase at a flow rate of 1 mL/min. The isocratic program was set at 20% (b) for 15 min. The mobile phases were filtered through 0.45 µm nylon membrane filters and degassed using an ultrasonic bath before analysis. The column temperature was maintained at 40°C and the injection volume was 1 µl. The wavelength was set at 280 nm.

Method validation

ICH guideline was employed for validation of the analytical method.[14] Limit of detection and limit of quantitation (LOQ) were calculated based on the residual standard deviation (SD) of a regression lines (σ) and the slope of the calibration curve (S) as 3.3 (σ)/S and 10 (σ)/S, respectively. The repeatability and intermediate precision were evaluated by analyzing 3 replicates of 3 different concentrations on 1-day and 3 consecutive days and expressed as percent relative SD (%RSD). The accuracy was determined by spiking (+)-catechin (50, 100, and 150 µg/mL) and (−)-epicatechin (50, 100, and 150 µg/mL) then percent recoveries were calculated. The specificity was evaluated by peak purity test. The robustness was determined for variations in flow rates (0.995, 1.000 and 1.005 mL/min) and variations in column temperature (39°C, 40°C and 41°C) and expressed as %RSD.

RESULTS

Microscopic examination

Pale catechu (*U. gambir* water extract) was small cylindrical in shape around 2.0–3.0 cm. The external was brown and internal was light brown or pale orange [Figure 1]. It was easy to break and bitter taste. Figure 2 was illustrated the whole plant of *U. gambir*.

Physico-chemical evaluations

Due to the ash contents, the physico-chemical parameters of pale catechu from 20 different sources could be divided into two classes [Table 1]. For pale catechu class I, the total ash, acid insoluble ash, loss on drying, moisture content, ethanol and water soluble extractive values were found to be 5.20 ± 0.19, 1.61 ± 0.17, 13.14 ± 0.10, 13.20 ± 1.07, 91.66 ± 5.16, and 44.59 ± 3.18 g/100 g of dry weight, respectively. For pale catechu class II, the total ash, acid insoluble ash, loss on drying, and moisture content, ethanol, and water soluble extractive values were found to be 29.80 ± 0.90, 21.27 ± 0.87, 10.41 ± 0.20, 9.35 ± 1.40, 60.20 ± 5.25, and 44.43 ± 2.99 g/100 g of dry weight respectively.

Thin-layer chromatography identification

TLC fingerprint of pale catechu was shown in Figure 3.

Antioxidant activities

The percentages of free radical scavenging activity between two classes were 75.58% ± 0.93% versus 75.02% ± 1.15%. FRAP values were of 0.35 ± 0.03 versus 0.23 ± 0.04 mol/m^3 $FeSO_4$/100 µg dry weight. The percentages of chelating activity were 3.16% ± 1.51% versus 2.69% ± 1.15%; while the chelating activity of EDTA standard was of 98.39%. The peroxidation inhibitions were 32.67% ± 2.58% versus 32.19% ± 3.68% [Table 1]. The percentage of free radical scavenging activity, chelating activity and FRAP value of (+)-catechin hydrate were found to be 82.66% ± 0.24%, 2.59% ± 1.87% and 0.542 ± 0.003 mol/m^3 $FeSO_4$/100 µg dry weight, respectively. The peroxidation inhibition of (+)-catechin hydrate (100 µg/mL) was found to be 18.12% ± 3.62%. Total phenolic, nontannin phenolic, and total tannin contents of commercial pale catechu classified as class I were 44.52 ± 0.15, 43.70 ± 0.23, and 0.82 ± 0.34 µg catechin equivalents/100 µg dry weight respectively. These phenolic contents of commercial black catechu classified as class II were 33.49 ± 0.17, 32.35 ± 0.34, and 1.14 ± 0.27 µg catechin equivalents/100 µg dry weight respectively [Table 1].

Table 1: Physical constants, anitioxidant activities and polyphenolic contents of pale catechu in Thailand*

	Class I	Class II
Physical constant (g/100 g (dry basis))		
Total ash	5.20±0.19	29.80±0.90
Acid insoluble ash	1.61±0.17	21.27±0.87
Loss on drying	13.14±0.10	10.41±0.20
Moisture	13.20±1.07	9.35±1.40
Ethanol extractives	91.66±5.16	60.20±5.25
Water extractives	44.59±3.18	44.43±2.99
Antioxidant activity		
DPPH inhibition (%)	75.58±0.93	75.02±1.15
FRAP value[a]	0.35±0.03	0.23±0.04
Ferrous ion chelation (%)	3.16±1.51	2.69±1.15
Beta-carotene bleaching (%)	32.67±2.58	32.19±3.68
Polyphenolic content		
Total phenolics[b]	44.52±0.15	33.49±0.17
Nontannin phenolics[b]	43.70±0.23	32.35±0.34
Total tannin[b]	0.82±0.34	1.14±0.27
(+)-catechin[c]	478.87±2.77	271.08±3.39
(−)-epicatechin[c]	Trace[d]	ND[d]

*The parameters were shown as grand mean±pooled SD. Samples were from 20 different sources throughout Thailand (5 class I, 15 class II). Each sample was performed in triplicate. [a]mol/m^3 ferrous sulfate/100 µg crude drug, [b]µg catechin equivalents/100 µg crude drug, [c]µg/mg crude drug, [d]Not detected/less than LOD or LOQ. LOD: Limit of detection, LOQ: Limit of quantitation, SD: Standard deviation, FRAP: Ferric reducing antioxidant power, DPPH: 1,1-diphenyl-2-picrylhydrazyl

Figure 1: Pale catechu crude drugs

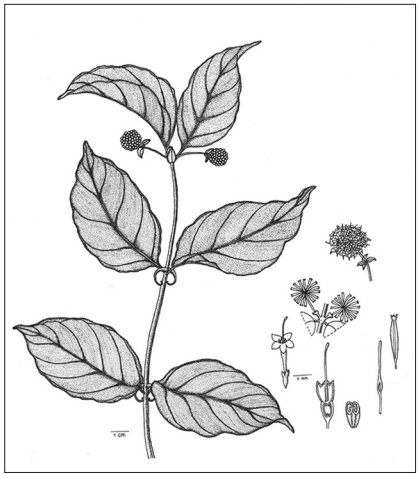

Figure 2: Whole plant of *Uncaria gambir*

High-performance liquid chromatograph analysis

HPLC chromatogram of pale catechu was illustrated in Figures 4-6 showed UV spectrum of (+)-catechin and (−)-epicatechin respectively. The results of HPLC analysis demonstrated that qualified pale catechu extracts were found to be rich source for (+)-catechin [Table 1]. However, (−)-epicatechin contents of most samples could not be determined quantitatively (<LOQ). The validity of catechins analyzed in pale catechu were summarized in Table 2.

DISCUSSION

Evaluation of pharmacognostic parameters revealed that the total ash and acid insoluble ash values of almost pale catechu samples were found to be high. Pale catechu monograph of Japanese pharmacopoeia specified that the total ash should be not more than 6.0 g/100 g and the acid insoluble ash should be not more than 1.5 g/100 g. It was suggested that the samples had adulterant problem. It might be adulterated with sand and other impurities.[15] The results were related to the previous studies in 1986 and 2009, which demonstrated that most samples of pale catechu in Thailand were substandard.[16,17] However, both classes of pale catechu showed promising antioxidant activities compared to (+)-catechin hydrate. This might be due to polyphenolic compounds in pale catechu, which were found to be nontannin phenolics. These results were in accordant with previous reports that a greater amount of phenolic contents leads to more potent radical scavenging effect.[3-6] The adulteration or contamination of commercial pale catechu in Thailand was in concern with the previous report.[16] This study revealed the difference in the quality of this crude drug especially (+)-catechin content. The results

Table 2: The method validation parameters of (+)-catechin and (−)-epicatechin

Parameter	(+)-catechin	(−)-epicatechin
Linearity	y=746.29x −2203.3	y=517.61x −652.07
R^2	0.9990	0.9989
Range (μg/mL)	5–200	5–200
Peak purity index	0.999	0.999
Accuracy: Percentage recovery	80.04-111.80	91.29-114.31
Precision (% RSD)		
Repeatability	0.16-0.68	0.26-0.79
Intermediate precision	1.44-1.86	1.23-2.71
LOD (μg/mL)	4.80	5.14
LOQ (μg/mL)	14.54	15.57
Robustness (% RSD)		
Retention time	0.58-0.96	0.58-1.09
Peak area	4.27-4.58	1.24-1.65

LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation

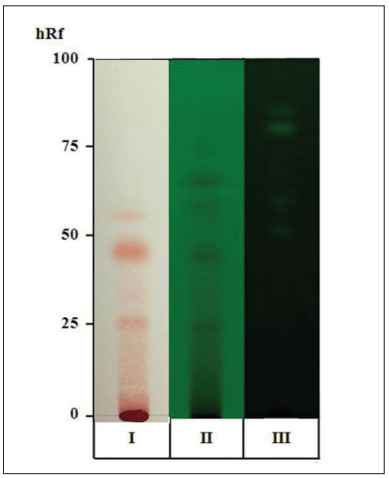

Figure 3: Thin-layer chromatography fingerprint of pale catechu with chloroform, ethyl-acetate and formic acid (3:6:1, v/v/v) as mobile phase (I: Detection with vanillin, II: Detection under ultraviolet light 254 nm, III: Detection under ultraviolet light 365 nm)

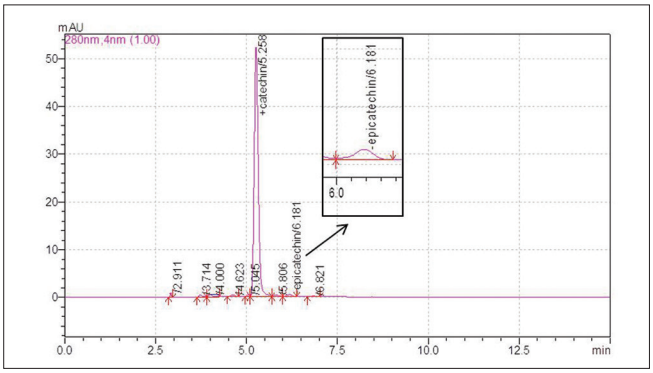

Figure 4: High performance liquid chromatograph chromatogram of pale catechu (isocratic elution: 80% water containing 0.1% formic acid and 20% acetonitrile containing 0.1% formic acid)

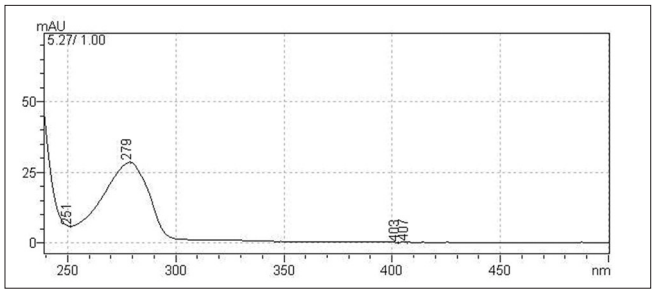

Figure 5: Ultraviolet spectrum of (+)-catechin

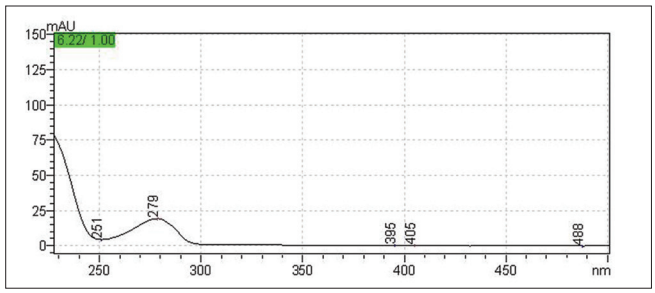

Figure 6: Ultraviolet spectrum of (−)-epicatechin

of HPLC analysis demonstrated that qualified pale catechu extracts were found to be a rich source for (+)-catechin. These findings were in accordant with the recent studies.[4,5]

CONCLUSION

The pharmacognostic investigations revealed the inferiority of pale catechu in Thai markets. The HPLC method showed good reliability for (+)-catechin and (−)-epicatechin quantification, which can be a tool to confirm the quality of pale catechu.

ACKNOWLEDGMENTS

The authors are gratitude to College of Public Health Sciences, Chulalongkorn University for necessary assistance and instrumental support.

Financial support and sponsorship
Nil.

Conflict of interest
There are no conflicts of interest.

REFERENCES

1. Taniguchi S, Kuroda K, Doi K, Inada K, Yoshikado N, Yoneda Y, *et al*. Evaluation of *gambir* quality based on quantitative analysis of polyphenolic constituents. Yakugaku Zasshi 2007;127:1291-300.
2. Taniguchi S, Kuroda K, Doi K, Tanabe M, Shibata T, Yoshida T, *et al*. Revised structures of gambiriins A1, A2, B1, and B2, chalcane-flavan dimers from *gambir* (*Uncaria gambir* extract). Chem Pharm Bull (Tokyo) 2007;55:268-72.
3. Amir M, Mujeeb M, Khan A, Ashraf K, Sharma D, Aqil M. Phytochemical analysis and *in vitro* antioxidant activity of *Uncaria gambir*. Int J Green Pharm 2012;6:67-72.
4. Anggraini T, Tai A, Yoshino T, Itani T. Antioxidative activity and catechin content of four kinds of *Uncaria gambir* extracts from West Sumatra, Indonesia. Afr J Biochem Res 2011;5:33-8.
5. Kassim MJ, Hussin MH, Achmad A, Dahon NH, Suan TK, Hamdan HS. Determination of total phenol, condensed tannin and flavonoid contents and antioxidant activity of *Uncaria gambir* extracts. Indones J Pharm 2011;22:50-9.
6. Apea-Bah FB, Hanafi M, Dewi RT, Fajriah S, Darwaman A, Artanti N, *et al*. Assessment of DPPH and α-glucosidase inhibitory potential of gambier and quantitative identification of major bioactive compound. J Med Plants Res 2009;3:736-57.
7. Neligan J. Medicines: Their Uses and Mode of Administration. 6th ed. London: Longman; 1864.
8. American Physician. The Electric and General Dispensatory: Comprehending a System of Pharmacy, Materia Medica and Receipts for the Most Common Empirical Medicine. Philadelphia: Mifflin and Parry Printers; 1827.
9. Kadam PV, Yadav KN, Narappanawar NS, Shivatare RS, Bhusnar HU, Patil MJ. Development of quality standards of *Terminalia catalpa* leaves. Pharmacogn J 2011;3:19-24.
10. Liu WJ. Traditional Herbal Medicine Research Methods. New Jersey: John Wiley and Sons; 2011.
11. World Health Organization. Quality Control Methods for Medicinal Plant Materials. Geneva: WHO Press; 2011.
12. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239:70-6.
13. Gupta D, Bleakley B, Gupta PK. Phytochemical analysis and antioxidant activity of herbal plant *Doronicum hookeri* Hook f. (*Asteraceare*). J Med Plants Res 2011;2:2736-42.
14. International Conference on Harmonization. Validation of analytical procedures: Text and Methodology Q2(R1) Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. [Last update on 2005 Nov; Last cited on 2015 Mar 17].
15. The Japanese Pharmacopoeia. The Japanese Pharmacopoeia Part II. 14th ed. Official Monographs; *Gambir* (English translation). Available from: http://www.jpdb.nihs.go.jp/jp14e/monographs2.html. [Last update on 2001 Apr 01; Last cited on 2015 May 08].
16. Dechatiwongse T, Jewvachdamrongkul Y. Quality determination of catechus. Bull Dept Med Sci 1986;28:79-91.
17. Mujarin K, Rattanakul P, Siripee J, Dechatiwongse T. Quality evaluation of catechu. FDA J 2009;16:39-46.