STANDARDIZED *SENNA ALATA* LEAF EXTRACT

Wandee Gritsanapan[1,*] and Peeranuch Mangmeesri[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
[2]Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

ABSTRACT: *Senna alata* (L.) Roxb. is a medicinal plant of which the leaves have long been used as a laxative and antifungal drugs. It is one of the plants recommended to be used in primary health care in Thailand and is included in the Thai traditional household drug list for laxative and antifungal herbal drugs. It is also listed in the Lists of National Herbal Drugs of Thailand. The leaves contain anthraquinones both aglycone and glycoside forms. The content of anthraquinones in the leaves are varied mainly according to cultivating locations and harvesting period. It is necessary to control the quantity of antraquinones in the leaves and leaf extracts of this plant for good quality of laxative and antifungal raw materials. This work was conducted to standardize 80% ethanolic extracts prepared by the appropriate maceration method of *S. alata* leaves collected from 10 different locations in 4 major parts i.e. the North, North-East, Central and South of Thailand. The extract ratio was 3-4:1, while the characteristics of the extracts were dark brown semi-solid with characteristic odour. The extract gave a positive Bontrager's test and its TLC-fingerprints showed 2 major anthraquinone components as rhein and aloe-emodin. Total anthraquinones content in the extract analyzed by UV-vis spectrophotometric method was not less than 2%w/w (average 2.48% w/w) calculated as rhein. Loss on drying of the extract should not more than 6%w/w while the extract was slightly soluble in water but freely soluble in 95% ethanol. For heavy metals contamination, the extracts should not contain arsenic, while lead, mercury and cadmium should not be found more than 2, 0.1 and 0.2 ppm, respectively. Total aerobic bacteria count and total yeast and mold count should not exceed 100 and 1000 cfu/g, respectively while none of pathogenic bacteria was found in the extract. Standardized *S. alata* leaf extracts could be used as a good quality raw material containing anthraquinones for laxative and antifungal pharmaceutical preparations.

Keywords: *Senna alata, Cassia alata,* Fabaceae, standardization, rhein, anthraquinone

INTRODUCTION: *Senna alata* (L.) Roxb. or *Cassia alata* L. is a medicinal plant in the family Fabaceae which has been known in Thai language as Chumhetthet. The English names are Candelabra bush and Ringworm bush[1)]. It is a native plant of South America and can be found widely in tropical region, up to 1500 m, on waste places often along ditches[2)]. In Indonesia, Philippines and Thailand, this plant can be found all over the countries, sometimes cultivated for medicinal purposes[3)]. After 3 months of planting, leaves are ready for harvest, but the best period for the best quality is about 6-7 months after planting[4)]. Fresh or dried leaflet of *S. alata* has been used as folk medicines in many countries for treatment of constipation, stomach pain, ringworm and skin diseases[5, 6)]. The leaf contains anthraquinones both aglycone and glycoside forms i.e. rhein, aloe-emodin, chrysophanol, glycosides of rhein, emodin, physcione and sennosides A, B, C, D[7-10)] while rhein is a major component[11)]. Gritsanapan *et al*[11)] investigated total anthraquinone glycosides content in the leaves of nine *Cassiae* i.e. *C. siamea, C. fistula, C. alata, C. surattensis* subsp. *surattensis, C. grandis, C. spectabilis, C. bakeriana, C. sophera* and *C. tora* collected in Summer, Winter and Rainy seasons from the Central areas of Thailand and found that the leaves of *C. alata* collected in Winter and Summer contain the highest amount (1.24% dry weight) of total anthraquinone glycosides. We also found that most of *Cassia* leaf samples containing the maximum content of anthraquinone glycosides are the samples collected in Summer (March-June) and Winter (November-February) seasons. *S. alata* is one of the plants recommended to be used in primary health care in Thailand and has been listed in Thai traditional household drug list for laxative and antifungal drugs. At present, *S. alata* is included in the List of Herbal Medicinal Products A.D. 2006 of Thailand[12)]. According to the Standard of ASEAN Herbal Medicine[4)] and Thai Herbal Pharmacopoeia (THP)[13)], *S. alata* leaves should contain not less

[*]To whom correspondence should be addressed.
E-mail: pywgs@mahidol.ac.th,
Tel +66 2644 8677-89 ext 1500, Fax +66 2644 8701

than 0.5 and 1.0 % dry weight of total hydroxyanthracene derivatives calculated as rhein-8-glucoside, respectively. The normal way of using *S. alata* for laxative is that 12 fresh or dried leaflets are coarsely cut, boil with 2 glasses of water until 1 glass of decoction is obtained and strain into a glass. Take the whole decoction as a single dose when needed. Another way, macerate 1-2 teabags of 3 g of dried powdered leaves in a cup of boiling water for 2-5 minutes, and take the infusion at bed time[14,15].

Extraction method and extracting solvent are important for quantity and quality of the extracts. Appropiate extraction method for each plant should be investigated in order to promote the highest amount of active components. Thus, this study was conducted to find out the appropriate extraction method for *S. alata* leaves to promote the 80% ethanolic extract containing the maximum amount of total anthraquinones and to standardize the extracts of *S. alata* leaves collected from 10 different locations in the North, North-East, Central and South of Thailand.

MATERIALS AND METHODS:

Plant Materials

The leaves of *S. alata* were collected from 10 different provinces in the North, North-East, Central and South of Thailand (Table 1) during the Winter (November-February) 2001-2002. The plant specimens were identified by comparison with the herbariums at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens (WSA010201-WSA010210) were deposited at Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. Each sample was air dried and then dried in a hot air oven at 50° C for 5 hours. The dried leaves were ground with an electronic mill and passed through a sieve (20 mesh).

Determination of Appropiate Extraction Method for *S. alata* Leaves

Due to the leaves of *S. alata* contain both anthraquinone aglycones which are soluble in ethanol, and glycosides which are soluble in water, so, 80%v/v was used as a solvent for extracting *S. alata* leaf samples.

Marceration: The powdered leaves of *S. alata* (10.0 g) were macerated with 80% ethanol (100 ml). The extraction was repeated until exhausted (tested by Borntrager's reaction) and the maceration extracts were combined, filtered and evaporated to dryness on a boiling water bath to yield a maceration crude extract (3.09 g).

Percolation: The powdered leaves of *S. alata* (10.0 g) were moistened with 80% ethanol (30 ml) for 15 minutes. The moistened material was put in a percolator and 80% ethanol was added. The percolation was adjusted at a rate of 1-3 ml/min until the extraction was exhausted. The extracts were combined, filtered and evaporated to dryness on a boiling water bath to yield a percolation crude extract (2.26 g).

Soxhlet extraction: The powdered leaves of *S. alata* (10.0 g) were extracted with 300 ml of 80% ethanol in a soxhlet apparatus. The extraction was continued until the extraction was exhausted. Each extract was then combined, filtered and evaporated to dryness on a hot water bath to yield a soxhlet crude extract (2.56 g).

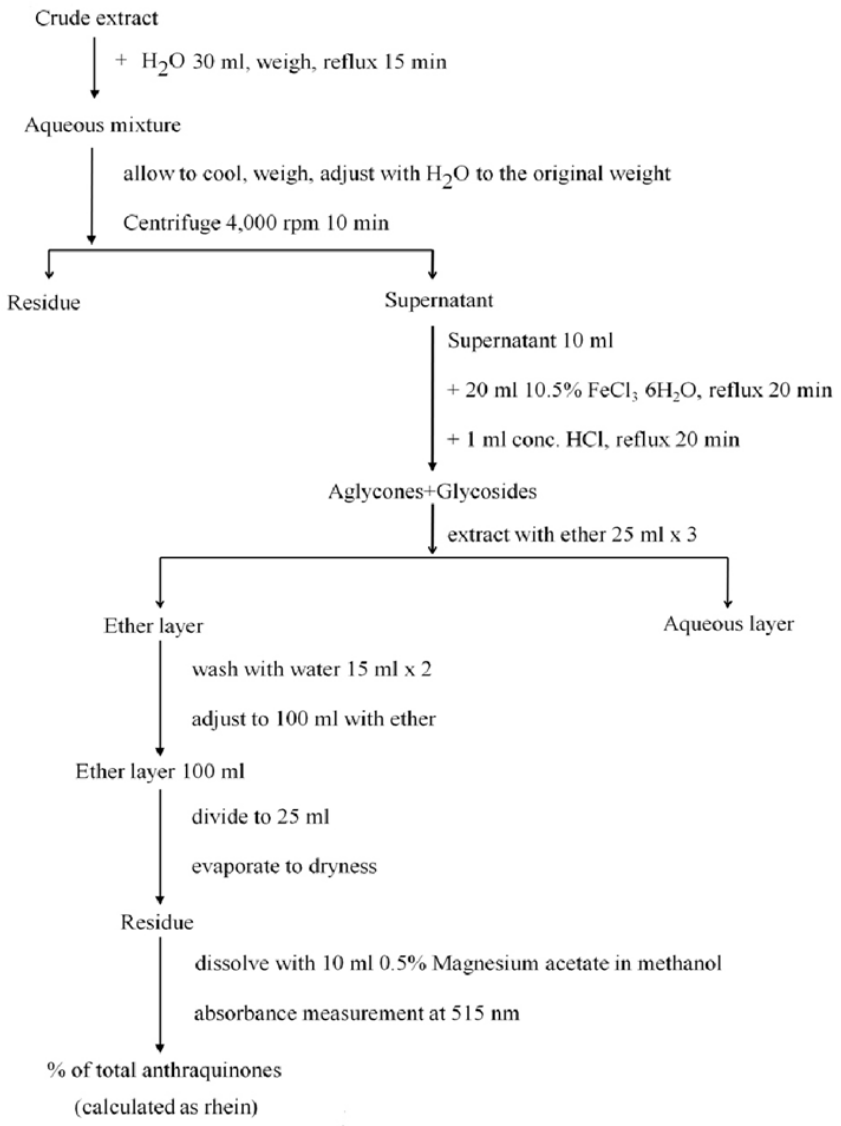
The extraction method which promoted the extract containing the maximum content of total anthraquinones would be choosen as the appropriate method for further extracting the leaf samples of *S. alata* collected from various locations.

Identification of Anthraquinones

Borntrager's reaction was used to detect anthraquinone aglycones in the extract. Hydrochloric acid (2M) was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with 10% potassium hydroxide solution. The upper aqueous layer becomes pink-red[16].

Quantitative Determination of Total Anthraquinones by a UV-visible Spectrophotometric Method in *S. alata* Leaf Extracts

The extract (0.10 g) was accurately weighed and dissolved in 30 ml of distilled water. The analysis procedure was followed the scheme1[4]. The content of total anthraquinones was determined by a validated UV-vis. Spectrophoto-



Scheme 1 Quantitative analysis of total anthraquinones[4)]

metric method[17)]. The UV absorbance was measured at 515 nm.

The content of total anthraquinones in the extract from each extraction method was calculated using the linear regression equation of a reference standard rhein. The contents were expressed as mean ± standard deviation (SD) (n=3).

Preparation of 95% Ethanol Extracts of *S. alata* Leaves Collected from Ten Different Provinces

Each sample of *S. alata* powdered leaves was exhaustively extracted with 80% ethanol by the appropriate extraction method (maceration). The combined extract was evaporated to yield a crude 80% ethanolic extract. The yield of crude extract was recorded and the extract ratio (weight of powdered drug : 1 g extract) was calculated.

TLC fingerprints

A 5.0 µL volume of each sample solution (20 mg/mL) was applied in form of band of width 8.0 mm on precoated silica gel aluminum plate 60 F_{254} (20x10 cm, E-Merck, Germany) using a Camag Limonat densitometer. The mobile phase consisting of ethyl acetate: methanol: water 100: 17:13 was used. Linear ascending development was carried out in 20x10 cm twin through glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The length of running was 8 cm. The development was carried out twice with

the same mobile phase to get good resolution of rhein. The TLC plate was dried with an air dryer. The anthraquinones were detected by spraying with 10% ethanolic KOH at room temperature (28° C). The positive result is a pink to red color of bands of anthraquinones detected in day light.

Determination of Total Anthraquinone Content in 80% Ethanolic Extracts of *S.alata* Leaves from 10 Different Provinces

The 80% ethanolic extracts of *S. alata* leaves from different locations were separately analyzed for total anthraquinone content, calculated as rhein, by UV-vis spectrophotometric method as described before. The analysis of each sample was done in triplicate and the content of total anthraquinone in each sample was reported as mean ± SD.

Physical and Chemical Properties of *S. alata* Leaf Extracts

Loss on drying determination

Loss on drying of each extract was determined according to the procedure described in Standard of ASEAN Herbal Medicine (1993). Each sample was done in triplicate and the average of weight lost on drying was reported.

Solubility of extracts

The solubility of each extract in 95% ethanol and distilled water was investigated at room temperature. The level of solubility was recorded according to USP XXVI criteria[18].

Heavy metal residues

Residues of heavy metals (Cd, Pb, As and Hg) in the extracts were determined according to the American Organization of Analytical Chemists (AOAC) official method of analysis[19].

Pesticide residues

Pesticides (organochlorines, organophosphates and pyrethroids) residues in the extracts were determined according to AOAC (2006).

Microorganism contamination

Total aerobic bacterial count and total fungi count were determined according to the method described in Standard of ASEAN Herbal Medicine (1993). Some pathogenic bacteria i.e. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp. and *Clostridium* spp. were also determined.

RESULTS AND DISCUSSION:

Determination of appropriate extraction method

Ethanol (80% v/v) was used as an extracting solvent for the leaf samples of *S. alata* due to both anthraquinone aglycones and glycosides can be soluble in this solvent, and ethanol is safe and not expensive. The extraction method which promoted the extract with maximum content of total anthraquinones (2.48 ± 0.20% w/w) was found to be the maceration while the extracts from the percolation and soxhlet extraction contained total anthraquinones 2.46 ± 0.31 and 2.13 ± 0.29%w/w, respectively. When compared various extraction methods concerning yields of crude extracts and total anthraquinone content in the extracts, maceration was found to be the appropriate extraction method. Thus, maceration was used for extracting *S. alata* leaves collected from 10 deferent locations.

Standardization of 80% ethanolic extracts of *S. alata* leaves

All 80% ethanolic extracts of *S. alata* leaves were dark brown semi-solid with characteristic odour. TLC fingerprints of all extracts showed the similar pattern while rhein and aloe-emodin were found to be major constituents with hRf values 39-44 and 74-79, respectively. The average extract ratio (crude drug: 1g crude extract) was 3.32:1 (3-4:1). The yields of crude extracts from the dried leaves were varied from 27.90 to 31.13% w/w (Table 1). The contents of total anthraquinones (total aglycones + total glycosides) calculated as rhein in all extracts were not much different and found in the range of 2.41 to 2.48 %w/w (average 2.45 % w/w). Compared to standardized senna leaf dry extract which contains not less than 5.5% and not more than 8.0% of hydroxy anthracene glycosides calculated as sennoside B[20], *Senna alata* leaf extract contained about 1/2 to 1/3 time of anthraquinones in the standardized senna extract. Loss on drying of the extracts was not more than 6% w/w (average 5.02 % w/w) (Table 2). All extracts were freely soluble in 95% ethanol and slightly

Table 1 Yield, extract ratio, and content of total anthraquinones in the ethanolic extracts and dried powder of *S. alata* leaves collected from various locations

Sample	Location/Part	Yield (%w/w)*	Extract ratio	Total antraquinones (%w/w)*	
				In extract	In dried powder
1	Bangkok/C	27.90±0.15	3.58:1	2.41±0.02	0.67±0.08
2	Phichit/C	29.85±0.08	3.35:1	2.47±0.03	0.74±0.02
3	Nongkhai/NE	30.95±0.10	3.23:1	2.48±0.30	0.77±0.04
4	Udonthani/NE	31.20±0.23	3.20:1	2.44±0.10	0.76±0.03
5	Maha sarakham/NE	30.94±0.12	3.23:1	2.43±0.05	0.75±0.12
6	Sukhothai/N	30.30±0.25	3.30:1	2.44±0.10	0.74±0.05
7	Phatthalung/S	29.82±0.17	3.35:1	2.47±0.08	0.74±0.10
8	Nakhon Si-thammarat/S	29.27±0.31	3.42:1	2.48±0.18	0.73±0.12
9	Ron Phibun-Nakhon Si-thammarat/S	31.13±0.29	3.21:1	2.47±0.19	0.77±0.17
10	Surat-Thani/S	29.73±0.17	3.36:1	2.47±0.20	0.73±0.15
average		30.11±0.18	3.32:1 (3-4:1)	2.45±0.12	0.74±0.09

*expressed as mean ±SD (n=3)
C = Central, NE =North-East, N = North, S = South

Table 2 Characteristics, loss on drying, heavy metal and pesticide residues, and microbial contamination of ethanolic extracts of *S. alata* leaves from 10 locations.

Test	Result	Limit allowed for dried herbal raw materials (1)	Limit allowed for food (2)
Color	dark brown		
Odour	characteristics		
Solubility in water	slightly soluble		
Solubility in 95% Ethanol	freely soluble		
Average loss on drying (%w/w)	5.02±0.34		
Test with Borntrager's	positive		
Heavy metal residues (ppm)			
Cd	< 0.17	< 0.3	-
Pb	< 2.00	< 10	1
As	ND	< 4	2
Hg	< 0.07	-	0.5
Pesticide residues (ppm)	ND	0.01-1	-
Microbial contamination (cfu/g)			
Total aerobic bacteria count	< 100	< 5.0×10^5/g	
Yeast and mold count	< 1000	< 5.0×10^3/g	
Enterobacteria	non	< 5.0×10^3/g	
E.coli	non	< 5.0/g	
P. auruginosa	non	non	
S. aureus	non	non/g	
Salmonella spp.	non	non/10g	
Clostridium spp.	non	non/10g	

(1) = Thai Herbal Pharmacopia Vol. I, 1995
(2) = Thai Food Acts, 1986
ND = not detectable

soluble in water. Due to no specific limit for herbal extracts on heavy metal and pesticide residues and microbal contamination,the limits allowed for herbal raw materials and foods were used as the guidances. The residues of heavy metals (Cd, Pb, As and Hg) in all extracts of *S. alata* leaves were below the allowed limits according to THP (Table 2). For pesticide residues, organochlorines, organophosphates and pyrethroids could not be detected in all extracts (Table 2). Microbial contamination in all extracts was found below the allowed limits according to THP (Table 2). Total aerobic bacterial and total fungi counts were less than 100 and 1,000 cfu/g, respectively while no pathogenic bacteria such as *S. aureus, E. coli, P. aeruginosa, Salmonella* spp. and *Clostridium* spp. were found in all the extracts.

CONCLUSION: Maceration was found to be appropiate for extraction *S. alata* leaves. 80% Ethanolic extracts of the leaves collected from 10 different locations in the North, North-East, Central and South of Thailand contained total anthra-quinones, calculated as rhein determined by UV-vis spectrophotometic method, not less than 2.0% w/w (average 2.45±0.12% w/w). TLC fingerprints of all extracts showed the same pattern which rhein and aloe-emodin were main constituents. Loss on drying of the extracts was not more than 6% w/w while the heavy metal

residues in the extracts were less than the limits allowed by ASEAN Herbal Medicine and THP. Pesticides were not detected in the extracts while microbial contamination was found below the limits allowed by THP for herbal raw materials, and no pathogenic bacteria was found in the extracts.

These specifications will be useful as guidances for quality assessment of the *S. alata* leaf extract as a good raw material for pharmaceutical laxative and antifungal preparations. With increasing doses, the extract of *S. alata* leaves could be used as an alternative source for senna (*Cassia senna* L.) leaf extract. This is the first time to report *S. alata* leaf extract specifications.

ACKNOWLEDGEMENT: This research was granted by The National Research Council of Thailand.

REFERENCES:

1. Smitinand T. 2001. Thai Plant Names. Revised ed., Bangkok: The Forest Herbarium, Royal Forest Department; p. 478.
2. Larsen K, Larsen SS, Vidal JE. 1984. Lguminosae, Flora of Thailand. vol. 4, part 1, Bangkok: The TISSTR Press; p.108-110.
3. Baansiddhi J, Pechaaply D, 1988. Botanical report of some Thai medicinal plants, part I, Bangkok: Department of Medical Sciences; p.8-9.
4. ASEAN Countries. 1993. Standard of ASEAN herbal medicine. vol. 1. Jakarta: Aksara Buana Printing; p.116-128.
5. Grosvenor PW, Gothard PK, Mc.William NC, Supriona A, Gray DO. 1995. Medicinal plants from Riau province, Sumatra, Indonesia Part I; uses. J Ethnopharmacol 45(2): 75-95.
6. Bhat RB, Etejere EO, Oladipo VT. 1990. Ethnobotanical studies from the Central Nigeria. Econ Bot 44(3): 382-390.
7. Villaroya LEM, Bernal-Santos R. 1976. A Chemical investigation of *Cassia al*ata Linn. Asian J Pharm 3 (1): 10-12.
8. Smith MR, Sodaquat A. 1979. Anthraquinone from the leaves of *Cassia alata* from Fiji. N J Sci. 22 (2): 123-126.
9. Rai PP. 1978. Anthracene derivatives in leaves and fruits of *Cassia alata*. Curr Sci 47(8): 271-272.
10. Rao JVLNS. 1975. Occurrence of kaempferol and aloe-modin in the leaves of *Cassia alata*, Curr Sci 44(20): 736-737.
11. Gritsanapan W, Phadungrakwitya R, Nualkeaw S, 2005. Investigation of alternative anthraquinone sources from *Cassia* spp. growing in Thailand. Proceeding of international conference on promotion and development of botanicals with international coordination. Kolkata: p. 120-126.
12. National Drug Committee. 2006. List of Herbal Medicinal Products A.D. 2006. Bangkok.: p.17-18.
13. Department of Medical Science, Ministry of Public Health. 1995. Thai Herbal Pharmacopoeia. Vol. 1**.** Bangkok: Prachachon Co. Ltd.; p. 17-23.
14. Ministry of Public Health, Thailand. 1984. Medicinal plants handbook. Bangkok: H.N. Ltd.; p. 47.
15. Ministry of Health Republic of Indonesia. 1981. Utilization of medicinal plants. 1st ed. p. 26.
16. Bruneton J. 1995. Pharmacognosy, Phytochemistry, Medical plants, Paris: Lavoisier Publishing; p. 351.
17. Sakulpanich A, Gritsanapan W. 2008. Extraction method for high content of anthraquinones from *Cassia fistula* pods. J Health Res 22(4): 167-172.
18. Authority of the United States Pharmacopoeial Convention. 2003. Solubility. USP XXVI, supplement 1. Asian ed. Maryland: p. 3015.
19. Association of Official Analytical Chemists. 2006. AOAC Official Method 986.15.
20. Council of Europe. 2001. European Pharmacopoeia. 4th ed., Strasbourg: p. 1885-1886.