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A Review Extraction and Standardization Techniques of Senna Leaves

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ABSTRACT

Senna alata (L) Roxb or *Cassia alataL* is a medicinal plant in the family Fabaceae which has been known in Thai language as Chumhetthet. Fresh or dried leaflet of *S. alata* has been used as folk medicines in many countries for treatment of constipation, stomach pain, and ringworm and skin diseases. 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.* In this paper overall study of the extraction process and standardization techniques of Senna leaves were reviewed successfully. In that various extraction method, the maceration extraction method was found the highest yield. The standardization process of senna leaves like LOD, Solubility extract, etc. was studied.

Keywords: Extraction process, *Senna alata*, Standardization techniques, Microwave assisted extraction

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 [1]. In Indonesia, Philippines and Thailand, this plant can be found all over the countries, sometimes cultivated for medicinal purpose [2]. 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 [3]. 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 [4]. The leaf contains anthraquinones both aglycone and glycoside forms i.e. rhein, aloeemodin, chrysophanol, glycosides of rhein, emodin, physcione and sennosides A, B, C, D [5] while rhein is a major component [6]. Gritsanapan et al. 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 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. According to the Standard of ASEAN herbal Medicine4) and Thai Herbal Pharmacopoeia (THP), *S. alata* leaves should contain not less than 0.5 and 1.0 % dry weight of total hydroxy anthracene derivatives calculated asrhein-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, macerate1-2 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 [7]. Extraction method and extracting solvent are important for quantity and quality of the extracts. The extraction method for each plant should be investigated in order to promote the highest number 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.

EXTRACTION METHOD

Maceration

The powdered leaves of *S. alata* (10.0 g) were macerated

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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 min. 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 chosen as the appropriate method for further extracting the leaf samples of *S. alata*.

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 min, then cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with10% potassium hydroxide solution. The upper aqueous layer becomes pink-red [8]. The extract (0.10 g) was accurately weighed and dissolved in 30 ml of distilled water. The content of total anthraquinones was determined by a validated UV-Visible Spectrophotometer. 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

Each sample of *S. alata* powdered leaf was exhaustively extracted with 80% ethanol by the appropriate extraction method (maceration). The combined extract was evaporated to yield a crude80% 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 percolated silica gel aluminum plate 60F254 (20 x 10 cm, E-Merck, Germany) using Camag Lim on at densitometer. The mobile phase consisting of ethyl acetate: methanol: water 100:17:13 was used. Linear ascending development was carried out in 20 x 10 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^{o}C). The positive result is a pink to red color of bands of anthraquinones detected in day light.

DETERMINATION OF TOTAL ANTHRAQUINONE

The 80% ethanolic extracts of *S. alata* leaves from different locations were separately analyzed for total anthraquinone content, calculated asrhein, by UV-Vis spectrophotometric method as described before.

Microwave-assisted extraction

It is a relatively new extraction technique, which utilizes microwave energy to heat the solvent and the sample and to increase the mass transfer rate of the solutes from the sample matrix into the solvent. The usage of microwaves for extracting plant constituents is still in infancy. 20 g of powdered senna leaves was extracted with 75 ml benzene for 15 min on electric shaker, filtered in vacuum and solvent distilled off. The left-over marc was dried at room temperature and extracted with 75 ml of 70% methanol for 25 min on electric shaker, filtered under vacuum.

The marc was re-extracted with 50 ml of 70% methanol for 15 min, filtered and the methanolic extracts combined. The methanolic extract was concentrated to 1/8th volume, acidified to pH 3.2 by adding HNO_3 with constant stirring. It was set aside for 15 min at 5°C, filtered and 1 g of anhydrous calcium chloride in 12 ml of denatured spirit was added with constant stirring. The pH of the solution was adjusted to 8 by addition of potassium hydroxide (KOH) and set aside for 15 min. above these procedures were study in three cases at different time for extraction on electric shaker. The precipitate obtained in all the three cases were collected, dried and weighed. The percentage yield was also calculated [9].

STANDARDIZATION OF SENNA LEAVES

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-loss 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 [10].

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.

Pesticide residues

Pesticides (organ chlorines, 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. *Staphylococcusaeruginosa, Salmonella* spp. and *Clostridium* spp were also determined.

CONCLUSION

In this paper overall study of the extraction process and standardization techniques of Senna leaves were reviewed successfully. In that various extraction method, the maceration extraction method was found the highest yield. The standardization process of senna leaves like LOD, Solubility extract, etc. was studied.

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