Although herbal medicines are generally perceived by the public as “natural” products, and therefore safe, there is voluminous evidence in the scientific literature that their use is not entirely risk-free. The risk is multiplied many fold if the products marketed are of poor quality, and do not contain ‘standardized’ levels of active compounds.

Standardization can be defined as the establishment of reproducible pharmaceutical quality by comparing a product with established reference substances, and by defining minimum and maximum amount of one or more compounds, or groups of compounds that are necessary in the product for efficacy and safety. In the field of phytomedicines, standardization may apply to the herbal raw materials, or to extracts prepared from raw materials. Many national and international agencies have prepared pharmacopoeial monographs of the most commonly used phytomedicines, and these monographs provide information on standards of active ingredients to be used in establishing the quality of herbal medicines.

The quality of herbal medicines is a major challenge because the content of active constituents in plants varies according to a large number of factors, and therefore generally the plants used in phytomedicine have a complex and inconstant chemical composition. The factors include the choice of the highest yielding plant species/variety, genetic composition of the plant, growth conditions (e.g. soil composition), geographical origin (climatic variation), age, and the specific parts of the plants harvested for processing.
Senna alata (L.) Roxb. or Cassia alata L. is a plant belonging to the family Leguminosae. S. alata leaves have long been traditionally used for the treatment of constipation and dermatophyte infections. Anthraquinone glycosides were demonstrated as the active constituents for the laxative properties [1], while the aglycones including aloe-emodin, rhein, emodin and chrysophanol exhibited antifungal activity [2,3].

The effects of plant parts, harvesting period, drying and storage on anthraquinone glycoside content in the leaves of Senna alata L. have been demonstrated [4]. Determination of anthraquinone glycoside content in the dried leaves of S. alata from Songkhla province, Thailand, collected from different positions of the plants and at different time period, indicated that anthraquinone glycosides markedly accumulated in the leaves collected from the upper positions of the plant (young and mature leaves). The anthraquinone glycoside content was lower for the leaves collected from the lower positions of the plant (older leaves). As regards the effect of harvesting period, the young leaves harvested in March and September, and the mature leaves harvested in June, gave higher amounts of anthraquinone glycosides. S. alata begins to blossom in November and fruits in December. In December, the anthraquinone glycosides were found to accumulate more in the flowers and pods than in the leaves. The effects of the drying method were also been demonstrated [4]. The temperature and the method of drying both play an important role in the quality of S. alata raw material preparation. The leaves that had been dried by using a hot air oven at 50°C contained a higher anthraquinone glycoside content [1.43% w/w dry weight (DW)] than those either using the hot air oven at 80°C (0.44% w/w DW), or those that had been dried in the sun for three days (0.95% w/w DW). This indicates that the anthraquinone glycosides are not stable at the high temperatures.
A simple, specific, precise, accurate, rapid, and reproducible HPLC method has been developed to quantify the anthraquinone content in *S. alata* leaf extracts [5]. The simultaneous quantitative determination for rhein, aloe-emodin, emodin and chrysophanol yield analyte data that provides useful marker information for the quality control of *S. alata* leaf extracts. The method involves the use of a TSK-gel ODS-80Tm column (5 µm, 4.6 x 150 mm) with a mixture of methanol and 2% aqueous acetic acid (70:30, v/v) as the mobile phase and UV detection at 254 nm. All four compounds were eluted within 30 minutes with good resolution. The parameters of linearity, repeatability, accuracy and specificity of the method were evaluated. The recovery of the method was 100.3 - 100.5% and linearity (correlation coefficient > 0.9998) was obtained for all anthraquinones. A high degree of specificity as well as repeatability and reproducibility (R.S.D. values less than 5%) were also achieved.

Based on HPLC analysis, only rhein (0.02% w/w) and aloe-emodin (0.03% w/w) are the major anthraquinone found in the methanol extract of *S. alata* leaves. A few different extraction solvents were tried to maximize the anthraquinone content in *S. alata* leaf extract [5]. *S. alata* leaves were extracted under reflux conditions using a mixture of HCl, FeCl₃ and water in methanol as the solvent. Water was used for extraction of anthraquinone glycoside, while HCl and FeCl₃ were used for hydrolysis and oxidation of anthraquinone glycoside as the aglycone. The concentrations of each component in methanol were varied in order to get a high yielding anthraquinone extract. The results revealed that emodin was observed when an acidic methanol was used for extraction, and 5%v/v HCl in methanol gave significant higher total anthraquinone content than those of 0, 3 and 10%v/v HCl in methanol. Variations of FeCl₃ concentration in methanol with 5% HCl were then examined as the extraction solvents. It was found that only aloe-emodin and emodin were observed in the extract after oxidization with FeCl₃. However, the content of total anthraquinones was increased when the concentration of FeCl₃ was increase to 5%w/v. Therefore, 5%w/v FeCl₃ in methanol with 5%v/v HCl was appropriately used for the extraction of the anthraquinones from *S. alata* leaves. This suggests that oxidization and hydrolysis of anthraquinone glycoside are required in the extraction process in order to increase anthraquinone content in *S. alata* extract. Although an efficient extraction method for dried *S. alata* leaves was established, extracts with low anthraquinone content (1.67% w/w) were still obtained.

To improve the potency of the antifungal activity of *S. alata* leaf extracts, the anthraquinone content of the extracts need to be increased, and the interfering compounds in the extracts (such as chlorophyll) need to be excluded from the extracts, in
particular to improve the physical appearance and stability of the extracts. Chromatographic methods were used to concentrate the anthraquinone in the leaf extracts of *S. alata*, as well as to greatly reduce the levels of other interfering compounds. Two chromatographic methods, an anion exchange and silica gel vacuum chromatography, were examined to improve the anthraquinone content in the leaf extracts [6]. Both methods were capable of increasing total anthraquinone content in the leaf extracts of *S. alata*. However, the extract that was isolated by silica gel vacuum chromatography gave higher content of total anthraquinones (16.7% w/w) than that isolated by anion exchange chromatography (9.6% w/w). The silica gel vacuum chromatographic method increased total content of anthraquinones in the extract up to 15-fold compared to level in the crude extract (1.1% w/w). In addition, isolation by silica gel vacuum chromatography was less time consuming than isolation by anion exchange chromatography. This indicates that silica gel vacuum chromatography is a preferable method for preparation of anthraquinone-rich *S. alata* extract. Average level of total anthraquinone content in the anthraquinone-rich *S. alata* extracts was 16% w/w.

Antifungal activity evaluation of the anthraquinone-rich *S. alata* extracts and the standard anthraquinones, aloe-emodin, rhein, emodin, chrysophanol against *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum* revealed that the anthraquinone-rich extract possessed antifungal activity against all tested dermatophytes with MICs between 15.62 - 250 µg/ml. This enriched extract showed the highest antifungal activity against *T. rubrum*, with an MIC 15.62 µg/ml. All tested dermatophytes were also completely inhibited by emodin and rhein at concentration between 1.95 - 1000 and 31.25 - 1000 µg/ml, respectively. Aloe-emodin exhibited the strongest antifungal activity against *T. rubrum* with MIC 0.98 µg/ml, but was not active against *T. mentagrophytes* and *M. gypseum* at the concentration up to 1,000 µg/ml. In contrast, chrysophanol was not active against all tested dermatophytes at concentrations up to 1,000 µg/ml. Although the antifungal activity of the anthraquinone-rich extract against *T. rubrum* was lower than that of aloe-emodin and emodin, its antifungal activities against *T. mentagrophytes* and *M. gypseum* were markedly higher than those of aloe-emodin and emodin. This is probably due to the synergistic effect of these two active compounds. Interestingly, the antifungal activity of the anthraquinone-rich *S. alata* leaf extracts suggests the potential use of the extracts as an antifungal agent. However, the extracts should be standardized to contain total anthraquinone content not less than 16% w/w of dry extracts.

References


