



Active Constituent-Rich Herbal Extracts for Development of Phytomedicine

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Plants are capable of synthesizing a large variety of secondary metabolites, i.e. glycoside, alkaloids, terpenoids, quinones, coumarins, flavonoids, phytosterols, saponins, and tannin, which contribute various pharmacological activities. Many conventional drugs or their precursors are now derived from plants. However, there is a difference between administering a pure phytochemical compound and the same chemical in a plant extract. Whether this “chemical complexity” is advantageous is still a matter of some debate. Synergy is an important aspect of treatments that involve medicinal plants.¹ In the context of chemical complexity it applies if the pharmacological effect of a chemical mixture is greater than the arithmetic sum of the effects of all individual components. This needs to be taken into account when considering the preparation of extracts with optimum levels of active constituent or “active constituent-rich extracts”. Moreover, most pure phytochemicals are not available commercially, and their isolation involves multi-stage, time-consuming and energy-intensive processes that require a large amount of toxic

organic solvents, ultimately increasing their production costs.

The extraction and fractionation processes are important steps for the preparation of an active constituent-rich extract with optimum pharmacological effect. The choices of a suitable solvent and extraction method as well as the selection of a simple and efficient fractionation method are capable of increasing the yield of active compounds in the plant extract. The present article describes some successful methods for preparation of active constituent-rich extracts, and their equivalent pharmacological activities compared with their major active constituents.

Rhinacanthin-rich extract from *Rhinacanthus nasutus* leaves

Rhinacanthus nasutus (Family Acanthaceae), named in Thai as “Tong Pan Chang”, has been used in Thai traditional medicine for treatment of tinea versicolor, ringworm, pruritic rash, abscess pain, and skin diseases. Rhinacanthin-C, rhinacanthin-D and rhinacanthin-N

(Figure 1), naphthoquinone compounds that can be isolated from *R. nasutus* leaves, exhibit antifungal, antibacterial, antiviral, anti-inflammatory, anti-allergic and cytotoxic activities.²⁻⁸

A reversed-phase HPLC has been established for the simultaneous determination of rhinacanthin-C, rhinacanthin-D and rhinacanthin-N in extracts from *R. nasutus* leaves. The method involves the use of a TSK-gel ODS-80Ts column (5 μ m, 4.6x150 mm) with a mixture of methanol and 5% aqueous acetic acid (80:20, v/v) as the mobile phase.² A few particular solvents, i.e. dichloromethane, chloroform, ethyl acetate, ethanol and methanol, have been found to efficiently extract rhinacanthins from *R. nasutus* leaf powder. Among the solvents used for extraction, one study found that ethyl acetate gave the highest content of total rhinacanthins (33.0% w/w of dried extract). This indicates that ethyl acetate is a suitable extraction solvent.²

Rhinacanthins are anionic compounds that can be enriched by anion exchange resins. The same study noted above found that an Amberlite[®] IRA-67 column was capable of enhancing the concentration of rhinacanthins in the obtained semi-purified extract. The content of total rhinacanthins in the rhinacanthin-rich extract was increased to 77.5% w/w compared to 37.4% w/w in the crude ethyl acetate extract. Moreover, interfering compounds, including chlorophyll and other pigments, were markedly excluded. The weakly basic anion exchanger, Amberlite[®] IRA-67, is therefore suitable for fractionation of *R. nasutus* leaf extract.²

For the current study, rhinacanthin-rich *R. nasutus* extract was prepared and standardized to contain total rhinacanthins not less than 70% w/w, and its antifungal activity against *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum* was evaluated and compared with those of the pure rhinacanthins. The antifungal activity

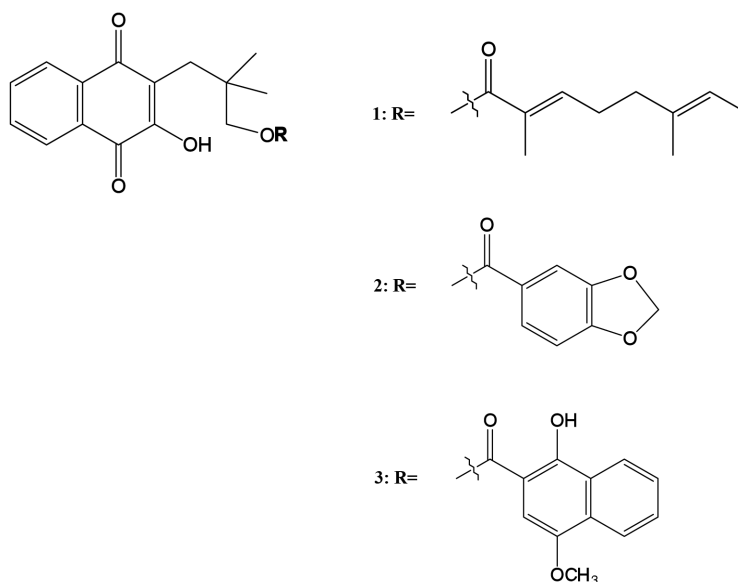


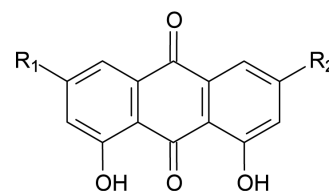
Figure 1 Chemical structures of rhinacanthin-C (1), rhinacanthin-D (2), and rhinacanthin-N (3).

of the rhinacanthin-rich *R. nasutus* extract was equal to that of rhinacanthin-C, the most potent antifungal constituent of *R. nasutus*.² In addition, the antimicrobial activity of the rhinacanthin-rich extract and rhinacanthin-C against *Streptococcus mutans*, *Propionibacterium acnes*, *Staphylococcus aureus* and *S. epidermidis* was determined and it was found that the antibacterial activity of the rhinacanthin-rich extract was almost equal to that of rhinacanthin-C.³ Moreover, the rhinacanthin-rich extract was studied for its analgesic and anti-inflammatory activities compared with rhinacanthin-C in experimental animals. The results confirmed that the rhinacanthin-rich extract and rhinacanthin-C possessed equivalent analgesic and anti-inflammatory efficacies in animal models.⁹ The standardized rhinacanthin-rich *R. nasutus* extract is therefore suitable for development of topical preparations for treatment of skin infectious diseases.

Anthraquinone-rich extract from *Senna alata* leaves

Senna alata (Fabaceae), called in Thai “Chumhetthet”, has been traditionally used for the treatment of dermatophyte infections. Anthraquinones, including aloe-emodin, rhein, emodin and chrysophanol (Figure 2), have been demonstrated to be active antifungals. Recently, extracts from *S. alata* leaves have had poor quality, containing a lower level of anthraquinones than the standard values given in the Thai Herbal Pharmacopoeia (1998). This has been a major problem in the production of the herbal medicine from *S. alata*. Although an efficient extraction method for dried *S. alata* leaves was established, extracts with low anthraquinone content were still being obtained.¹⁰ To improve the potency of the antifungal activity of *S. alata* leaf extracts, the anthraquinone content of the extract needed to be increased, and the interfering compounds in the extracts, such as chlorophyll, needed to be excluded

from the extracts, in particular to improve the physical appearance and stability of the extracts.



Aloe-emodin	: R ₁ =CH ₂ OH	R ₂ =H
Chrysophanol	: R ₁ =CH ₃	R ₂ =H
Emodin	: R ₁ =CH ₃	R ₂ =OH
Rhein	: R ₁ =COOH	R ₂ =H

Figure 2 Chemical structures of anthraquinones.

Dried *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 any anthraquinone glycoside to the aglycone form. The concentrations of each component in the solvent were varied in order to get a high yielding anthraquinone extract. Finally, a solution of 5% v/v hydrochloric acid, 5% w/v ferric chloride and 15% v/v water in methanol was selected as the extraction solvent to extract anthraquinone from *S. alata* leaves. This indicates that oxidization and hydrolysis of anthraquinone glycoside are required for an optimum process of extracting anthraquinone from *S. alata* extract.¹⁰

Chromatographic methods are used to concentrate the anthraquinone in the crude 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 studied to attempt to increase the anthraquinone content

in the leaf extract. Both methods improved the total anthraquinone content in the extracts. However, the extract that was isolated by silica gel vacuum chromatography gave a higher content of total anthraquinones than that isolated by anion exchange chromatography. The silica gel vacuum chromatographic method increased the total content of anthraquinones in the extract by up to 15-fold compared to the levels in the crude extract. 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 improving the anthraquinone content in *S. alata* leaf extracts. The yield of the anthraquinone-rich extract was 0.7% w/w compared to the weight of dried leaf powder. The total anthraquinone content in the extract was 16.2% w/w.¹¹

Evaluation of the antifungal activities of the anthraquinone-rich *S. alata* extract and the standard anthraquinones, aloe-emodin, rhein, emodin, chrysophanol, against *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum* established that the anthraquinone-rich extract possessed antifungal activity against all tested dermatophytes with MIC values of between 15.6–250.0 µg/mL. This enriched extract showed the highest antifungal activity against *T. rubrum*, with an MIC value of 15.6 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 a synergistic effect between these two active compounds.

These results confirm the potential of the anthraquinone-rich *S. alata* leaf extracts for use as an effective antifungal preparation, and suitable for developing formulations for treatment of fungal infections. However,

the extracts should be standardized to have a total anthraquinone content of not less than 16% w/w.¹¹

Ellagic acid-rich extract from pomegranate fruit peel

Pomegranate fruit peel, a by-product of the pomegranate juice industry, is a rich source of hydrolyzable tannins belonging to the ellagitannins. A wide range of clinical applications of pomegranate fruit peel extract for the treatment and prevention of cancer, as well as other diseases, where chronic inflammation is believed to play an essential etiologic role, has been suggested.¹² Pomegranate fruit peel extract is also a potential antioxidant agent for nutraceutical and cosmetic applications.¹³ Ellagic acid (Figure 3) is considered to be a suitable indicative marker for standardization of the fruit peel extract^{14,15} and as a biomarker for human bio-availability studies involving consumption of ellagitannins from any food source.¹⁶ In addition, ellagic acid also has other benefits; it has anti-inflammatory,¹⁷ anti-allergy,¹⁸ and anti-cancer properties,^{19,20} it reduces microbial growth,²¹ it combats many toxic effects caused by phospholipase A₂²² and it protects against testicular toxicity.²³ Thus, a method for enriching, extracting and measurement of the ellagic acid content of pomegranate fruit peel extract is needed.

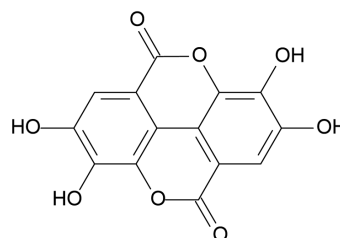


Figure 3 Chemical structure of ellagic acid.

Various extraction solvents were determined to attempt to increase the ellagic acid concentration in pomegranate fruit peel extract. Considering the polarity of ellagic acid, a combination of water and methanol was first chosen as extraction solvents. A solvent of 10% v/v water in methanol gave significantly higher ellagic acid content than other extraction solvents. The obtained extract was further purified by partitioning between water and a few organic solvents, i.e. ethyl acetate, *n*-butanol and 50% v/v *n*-butanol in ethyl acetate. The ethyl acetate fraction showed the highest ellagic acid content, as well as the highest antioxidant activity.¹⁵ Furthermore, after fractionation of the pomegranate fruit peel extract using liquid-liquid extraction between ethyl acetate and 2% aqueous acetic acid, an ellagic acid-rich extract was obtained from the ethyl acetate fraction. This method was capable of increasing the ellagic acid content in the extracts from 7.1% w/w to 13.6% w/w as well as producing a significant increase in its antioxidant activity. According to the satisfactory antioxidant activity of the ellagic acid rich pomegranate it was decided that extracts to be used should be standardized to have an ellagic acid content of not less than 13% w/w.²⁴

Antibacterial activity determination of the standardized ellagic acid-rich extract (ellagic acid content of not less than 13% w/w) using the disc diffusion method revealed that the standardised extract (2 mg/disc) possessed an inhibitory effect against Gram-positive bacteria including *Propionibacterium acnes*, *Staphylococcus aureus* (both standard and isolated strains) and *Staphylococcus epidermidis*. The extract exhibited a satisfactory inhibitory effect on *P. acnes*, with an MIC value of 15.6 µg/mL, and *S. aureus* and *S. epidermidis* had MIC values of 7.8–15.6 and 7.8 µg/mL, respectively. In contrast, all tested bacteria were resistant to the pure ellagic acid at concentrations of up to 2 mg/mL.²⁵

Evaluation of inhibitory activity against nitric oxide (NO) production revealed that the standardized ellagic acid-rich extract possessed marked anti-NO effects, with an IC₅₀ value of 10.7 µg/mL. Its potency was the same as that for L-nitroarginine, a NO synthase inhibitor.²⁵ Determination of anti-allergic activity using an inhibitory assay on the release of β-hexosaminidase from RBL-2H3 cells revealed that the standardized ellagic acid-rich extract and ellagic acid possessed a marked inhibitory activity with IC₅₀ values of 20.9 and 4.3 µg/mL, respectively. Therefore, both the standardized ellagic acid-rich extract and ellagic acid are potent anti-allergic agents when compared to the inhibitory effect of ketotifen fumarate (IC₅₀ of 20.2 µg/mL), a positive control. In addition, the standardized extract exhibited moderate cytotoxicity to human keratinocyte cells with a CC₅₀ value of 33.6 µg/mL.²⁵

In addition, evaluation of the topical anti-inflammatory and analgesic effects of the standardized ellagic acid-rich extract and ellagic acid using mouse models of acute and chronic skin inflammation as well as contact dermatitis showed that the ellagic acid-rich extract and ellagic acid possessed equivalent anti-inflammatory and analgesic effects. Their topical formulations are therefore promising therapies for inflammatory diseases such as cutaneous inflammation, arthritis and contact dermatitis.^{26–28}

An *in vivo* investigation of the wound healing potential of the standardized ellagic acid-rich extract and ellagic acid using rat dermal wound models showed that both the ellagic acid-rich extract and its equivalent amount of ellagic acid increased the tensile strength of the incision wound by up to 35.4 and 31.8%, respectively. The standardized extract accelerated wound contraction of both excision and burn wound models, while ellagic acid was effective only at 0.6% in these two wound models. Moreover, the ellagic acid-rich extract enhanced the synthesis of collagen and inhibited neutrophil infiltration

dose dependently, while ellagic acid was not effective in increasing collagen accumulation and its inhibitory effect on neutrophil infiltration was milder. These results indicated that the standardized ellagic acid-rich extract is a promising agent for wound healing effect and this effect is better than its marker compound, ellagic acid.²⁹

These results support the potential use of the standardized ellagic acid-rich pomegranate extract for antibacterial, anti-inflammatory and analgesic, wound healing, and anti-allergic purposes.

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