

Title	EVALUATION OF ANTIFUNGAL ACTIVITY OF <u>ASPARAGUS RACEMOSUS</u> WILLD. ROOTS
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ABSTRACT

Asparagus racemosus Willd (Asparagaceae family), known as Shatavari, is an important medicinal plant in Ayurvedic medicine. The major active constituents of *A. racemosus* roots are steroidal saponins such as shatavarins I-IV. The aims of this study are to compare the *A. racemosus* extracts using different solvents and to investigate their antifungal activities.

A. racemosus roots collected from 3 provinces in Thailand were successively extracted with the series of solvents i.e. hexane, ethanol and water. Moreover, the method for enriching saponins in the extract was applied. The profiles of chemical constituents in *A. racemosus* were determined by thin layer chromatography and the amounts of saponin equivalent to shatavarin IV in the extracts were analyzed by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody (MAb) against shatavarin IV. The extracts were tested for antibacterial and antifungal activities by disc diffusion and broth microdilution methods. The synergistic effect of *A. racemosus* extracts and antifungal agents i.e. ketoconazole and zinc pyrithione were performed using checkerboard synergy test. The stability of the extracts after storing at 50 °C for 30 days was studied.

The quantitative analysis using ELISA showed that the extract collected from Rayong possessed the highest saponin content among 3 sources of *A. racemosus*. In addition, when comparing the levels of saponin obtained from different extraction

methods, high saponin contents were obtained in the ethanolic extract (AR-E) and the saponin enriched extract (AR-En) (7.43 ± 0.45 and $38.34 \pm 1.42\%$, respectively). It is noted that saponin enrichment method could increase the level of saponin from the solvent extraction method for at least 5 times. Moreover, AR-E and AR-En showed an antifungal activity against *Candida albicans*, *Malassezia furfur* and *M. globosa* at the concentration of 1 mg/disc while the extracts using the other solvents showed no inhibitory effect. The results from broth microdilution method showed that AR-E had minimum inhibitory concentration (MIC) values against the 3 fungi in the range of 2-25 mg/ml. Interestingly, AR-En had stronger inhibition effect on the fungi expressed in the lower MICs in comparison with AR-E (0.10 mg/ml for *C. albicans*, 0.40 mg/ml for *M. furfur* and 0.20 mg/ml for *M. globosa*). The synergistic effects of AR-E and AR-En with antifungal agents i.e. ketoconazole and zinc pyrithione against *M. furfur* and *M. globosa* were studied. However, no synergistic effect was observed. In addition, the stability study showed that after storing at 50 °C for 30 days, the antifungal activity and saponin level of AR-E had no change from the original extract.

In conclusion, we succeeded to prepare the extracts of *A. racemosus* roots with antifungal activity using ethanol extraction and saponin enrichment method. Both extracts could be standardized for saponin content using ELISA. The extracts might be used as ingredients in antifungal products such as antidandruff formulation or vaginal cleansing soap.