EVALUATION OF ANTIFUNGAL ACTIVITY OF *ASPARAGUS RACEMOSUS* WILLD. ROOTS

CHURANYA ONLOM

A Thesis Submitted to the Graduate School of Naresuan University
In Partial Fulfillment of the Requirements
for the Master of Science Degree
in Pharmaceutical Chemistry and Natural Products (International Program)
May 2011

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This thesis entitled "Evaluation of Antifungal Activity of *Asparagus racemosus* Willd. Roots" submitted by Churanya Onlom in partial fulfillment of the requirements for the Master of Science Degree in Pharmaceutical Chemistry and Natural Products (International Program) is hereby approved.

..... Chair
(Associated Professor Jarupa Viyoch, Ph.D.)

..... Committee
(Associated Professor Kornkanok Ingkaninan, Ph.D.)

..... Committee
(Associated Professor Neti Waranuch, Ph.D.)

..... Committee
(Sophit Khanthawong, Ph.D.)

..... Committee
(Professor Malyn Chulasiri, Ph.D.)

Approved

.....
(Assistant Professor Kanungnit Pupatwibul, Ph.D.)
Dean of Graduate School
18 May 2011

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Churanya Onlom

Title	EVALUATION OF ANTIFUNGAL ACTIVITY OF ASPARAGUS RACEMOSUS WILLD. ROOTS
Author	Churanya Onlom
Advisor	Associate Professor Kornkanok Ingkaninan, Ph.D.
Co-Advisor	Associate Professor Neti Waranuch, Ph.D. Sophit Khanthawong, Ph.D.
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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 ± 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 pyrithyone 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.

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ABBREVIATIONS

A. racemosus	=	*Asparagus racemosus*
AR 1-A	=	*Asparagus racemosus* aqueous-A extract from Tak
AR 2-A	=	*Asparagus racemosus* aqueous-A extract from Kanchanaburi
AR 3-A	=	*Asparagus racemosus* aqueous-A extract from Rayong
AR 1-B	=	*Asparagus racemosus* aqueous-B extract from Tak
AR 2-B	=	*Asparagus racemosus* aqueous-B extract from Kanchanaburi
AR 3-B	=	*Asparagus racemosus* aqueous-B extract from Rayong
AR 1-E	=	*Asparagus racemosus* defatted ethanolic extract from Tak
AR 2-E	=	*Asparagus racemosus* defatted ethanolic extract from Kanchanaburi
AR 3-E	=	*Asparagus racemosus* defatted ethanolic extract from Rayong
AR 3-En	=	*Asparagus racemosus* saponin enriched extract from Rayong
AR 1-H	=	*Asparagus racemosus* hexane extract from Tak
AR 2-H	=	*Asparagus racemosus* hexane extract from Kanchanaburi
AR 3-H	=	*Asparagus racemosus* hexane extract from Rayong
AR 1-S	=	*Asparagus racemosus* sedement extract from Tak
AR 2-S	=	*Asparagus racemosus* sedement extract from Kanchanaburi
AR 3-S	=	*Asparagus racemosus* sedement extract from Rayong
C. albicans	=	*Candida albicans*
°C	=	degree Celsius
ELISA	=	Enzyme-linked immune-sorbent assay
E. coli	=	*Escherichia coli*
g	=	gram
hr	=	hour
KTZ	=	Ketoconazole
LC-MS/MS	=	Liquid chromatography-tandem mass spectrometry
M. furfur	=	*Malassezia furfur*
M. globosa	=	*Malassezia globosa*
MIC	=	Minimum inhibitory concentration

ABBREVIATIONS (CONT.)

MFC	=	Minimum fungicidal concentration
S. aureus	=	*Staphylococcus aureus*
SDA	=	Sabouraud dextrose agar
St-IV	=	Standard shatavarin IV
µl	=	micro liter
ZPT	=	Zinc pyrithione
mg	=	milligram
min	=	minute
mL	=	milliliter
pH	=	power of hydrogen ion concentration
Ps. aeruginosa	=	*Pseudomonas aeruginosa*
UV	=	ultraviolet
w/v	=	weight by volume
w/w	=	weight by weight