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ANTI-OXIDATIVE STRESS ACTIVITY OF PHIKUD NAVAKOT EXTRACT IN *SACCHAROMYCES CEREVISIAE*

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INTRODUCTION

Phikud Navakot is a polyherbal formula in Yahom included in List of Herbal Medicinal Products A.D. 2012. Yahom is primarily used as cardiogenic agent to increase cardiac output according to Thai traditional knowledge. Scientific evidence demonstrating biological activity of Phikud Navakot has to be evaluated. Phikud Navakot comprises an equal weight of nine crude drugs namely Kot Soa [root of *Angelica dahurica* (Fisch.) Benth. & Hook. f.], Kot Khamao [rhizome of *Atractylodes lancea* (Thunb.) DC.], Kot Hua Bua (rhizome of *Ligusticum chuanxiong* Hort.), Kot Chiang [root of *Angelica sinensis* (Oliv.) Diels], Kot Chulalumpa (aerial part of *Artemisia pallens* Wall. ex Besser), Kot Kradook [rhizome of *Saussurea costus* (Falc.) Lipsch.], Kot Kan Prao (rhizome of *Picrorhiza kurrooa* Royle ex Benth.), Kot Pung Pla (gall of *Terminalia chebula* Retz.), and Kot Jatamansi [root and rhizome of *Nardostachys jatamansi* (D. Don) DC.]. Active compounds isolated from these herbs were found to exhibit antioxidant activity, for example, picroliv, picroside-I and kutkoside from *P. kurrooa*¹, and ferulic acid from root of *A. sinensis*². Oxidative stress is one contributing factor in development of several pathophysiological conditions including cardiovascular diseases, neurodegenerative diseases, cancer and aging³. Anti-oxidative stress activity at the cellular level of Phikud Navakot should be determined to support its traditional use. *Saccharomyces cerevisiae* is a model eukaryotic organism widely used in the study of cellular processes^{4,5}. The present study was aimed to investigate the effect of Phikud Navakot extract against oxidative stress induced by hydrogen peroxide in yeast model.

MATERIALS AND METHODS

Preparation of Phikud Navakot extract Each crude drug was ground into coarse powder. Equal amount (weight) of the 9 crude drugs were combined and macerated in 10 times by weight of 80% ethanol for overnight. Extraction was done by heating at 100 °C for 3 h and the liquid phase was separated by filtration. The solid phase was re-extracted with the same procedure. The two filtrates were combined and the ethanol was removed by evaporation under reduced pressure to yield the concentrate Phikud Navakot extract (NVK).

Preparation of yeast cells *S. cerevisiae* ATCC 26108 was grown in yeast peptone dextrose (YPD) broth [1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) glucose] at 30 °C with 160 rpm shaking for 72 h. Yeast cells were collected by centrifugation at 3,000 g for 3 min, washed 3 times with phosphate buffer saline (PBS) and suspended in PBS to yield 2x10⁸ CFU/mL. The yeast suspension was incubated at 30 °C, 160 rpm for 1 h before determining the IC₅₀ value induced by H₂O₂ and anti-oxidative stress analysis.

Determination of IC₅₀ of hydrogen peroxide The yeast suspension was diluted in PBS to yield 1x10⁴ CFU/mL which was further aliquoted into 2-mL screw cap microcentrifuge tubes. H₂O₂ (100 µL) was added to obtain the final concentrations of 0.5, 1.0, 2.0, and 3.0 mM. The tubes were loosely capped and incubated at 30 °C with 650 rpm shaking (Thermomixer Comfort, Eppendorf®) for 1 h. The cell viability was determined by drop plate method on YPD agar. The agar plates were incubated at 30 °C for 48 h and the colonies were counted. Percent cell survival was plotted against H₂O₂ concentration and the IC₅₀ value was determined as the concentration of H₂O₂ resulted in 50% survival.

Anti-oxidative stress assay The 950-µL aliquots of NVK in PBS at various concentrations were mixed with 50 µL of the 2x10⁸ CFU/mL yeast cell suspension in PBS pre-conditioned at 30°C, 160 rpm for 1 h as described above. Quercetin (0.1 mM) was used as a positive control. Sample from each tube was taken for viable count to determine the CFU at the beginning (T₀) of NVK and quercetin treatments. A 200-µL aliquot of the remaining mixture from each tube was transferred to 2-mL screw cap microcentrifuge tube and incubated at 30 °C, 650 rpm for 1 h. After 1 h incubation, the treated yeast cells were diluted 1,000

times in PBS and mixed with an equal volume of $2 \times IC_{50}$ H_2O_2 and incubated at $30^\circ C$, 650 rpm for 1 h. Percent cell survival was determined as described above.

Statistical Analysis Data are expressed as mean \pm SD. Student's *t*-test was used to compare the significant difference between two groups.

RESULTS

Determination of IC_{50} value of H_2O_2 against stationary phase *S. cerevisiae* There was a linear relationship between the killing activity (expressed as % survival of the yeast cells) and the log concentration of H_2O_2 as shown in Figure 1. The IC_{50} value which is the concentration of H_2O_2 that killed half of the *S. cerevisiae* cells calculated from the graph was 1.6 mM.

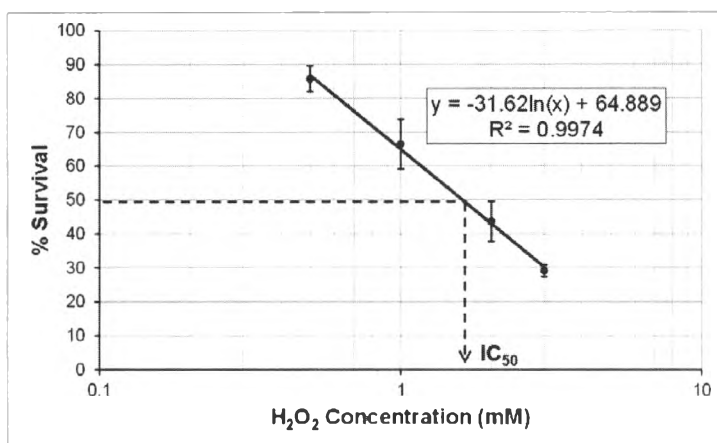


Figure 1 Percent survival of *S. cerevisiae* after treated with various concentrations of H_2O_2 for 1h. The IC_{50} value calculated from the graph is shown. Results were represented as mean \pm SD from triplicate determinations.

Protective effect of NVK against H_2O_2 -induced oxidative stress in *S. cerevisiae* *S. cerevisiae* cells were pretreated with 5 to 1000 $\mu g/mL$ of NVK (or 34 $\mu g/mL$ quercetin, a known antioxidant) and then challenged with H_2O_2 at the IC_{50} . The results are shown in Figure 2. Pretreating with 5 or 10 $\mu g/mL$ of NVK had no effect on yeast cell survival as compared with the control experiment. NVK (20 and 25 $\mu g/mL$) could increase the % survival of the yeast cells from 51% (control) to 59% and 60%, respectively. However, these were not statistically significant different from the control. NVK (30 $\mu g/mL$) significantly increased the survival of the yeast cells to 63%. Increasing NVK concentration to 50 $\mu g/mL$ resulted in disappearance of the protective effect. Importantly, the NVK at the concentration of 100 $\mu g/mL$ and higher concentration-dependently became toxic to the cells.

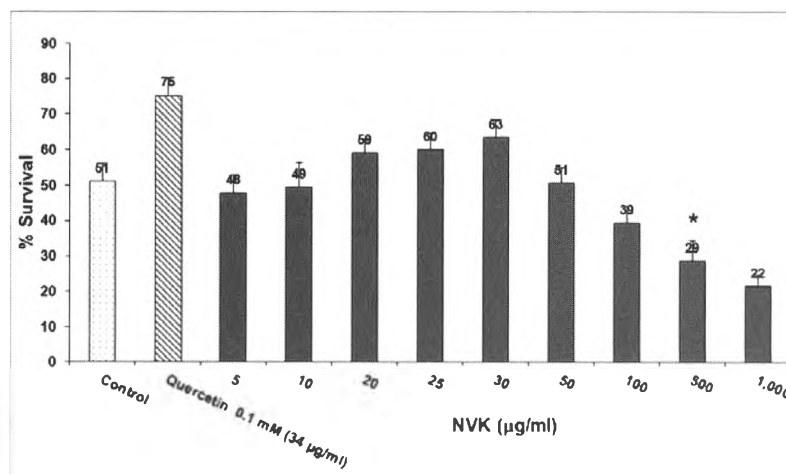


Figure 2 Percent survival of *S. cerevisiae* pretreated with various concentration of NVK for 1 h before treated with 1.6 mM H_2O_2 for 1 h. Quercetin was used as a positive control. Results were expressed as mean \pm SD from triplicate determinations. * indicates statistically significant difference from the control ($p < 0.05$).

DISCUSSION

S. cerevisiae at stationary phase is a useful model organism for oxidative stress studies. Stationary yeast cells and most eukaryotic multicellular organism share a number of important characteristics including quiescent phase of cell cycle (G_0)⁶. Cultivation of yeast cells in YPD broth with shaking at 30 °C for 72 h could reach saturation (2×10^8 cells/mL), suggesting that cells were in stationary phase as previously report⁷. NVK (30 μ g/mL could protect yeast cells from H_2O_2 -induced oxidative stress killing, showing anti-oxidative stress activity of NVK. However treatment of NVK at 3-fold higher concentration significantly decrease percent survival of yeast cells in a concentration dependent manner. Anti-oxidative stress activity of NVK in yeast cells found in this study suggested that Phikud Navakot in Yahom might ameliorate oxidative stress in human leading to the cardiotoxic activity of Yahom.

CONCLUSION

NVK at the optimum concentration increases resistance of yeast cells against H_2O_2 -induced oxidative stress. Further in-depth and detailed studies, such as proteomic analysis of NVK-treated yeast cells, should be further carried out to elucidate the target(s) and exact mechanism of this cytoprotective effect of NVK. Nevertheless, our finding of the NVK toxicity in yeast cells implies that Phikud Navakot at high dose might be toxic to human and should be further clarified. Special precaution of Phikud Navakot ingestion should be taken for not taking high amount or not regularly taking for a long period.

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REFERENCES

1. Chander R, Kapoor NK, Dhawan BN. 1992. Picroliv, picroside-I and kutkoside from *Picrorhiza kurrooa* are scavengers of superoxide anions. *Biochem Pharmacol* 44(1):180-3.
2. Cheng CY, Ho TY, Lee EJ, Su SY, Tang NY, Hsieh CL. 2008. Ferulic acid reduces cerebral infarct through its antioxidative and anti-inflammatory effects following transient focal cerebral ischemia in rats. *Am J Chin Med* 36(6):1105-19.
3. López-Alarcón C, Denicola A. 2013. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. *Anal Chim Acta* 763:1-10.
4. Petranovic D, Nielsen J. 2008. Can yeast systems biology contribute to the understanding of human disease? *Trends Biotechnol* 26:584-90.
5. Müller B, Grossniklaus U. 2010. Model organisms – a historical perspective. *J Proteomics* 73: 2054-63.
6. Zakrajšek T, Raspor P, Jamnik P. 2011. *Saccharomyces cerevisiae* in the stationary phase as a model organism - characterization at cellular and proteome level. *J Proteomics*. 74(12):2837-45.
7. Della Croce C, Bronzetti G, Cini M, Caltavuturo L, Poi G. 2003. Protective effect of lipoic acid against hydrogen peroxide in yeast cells. *Toxicol in Vitro* 17(5-6):753-9.