ประสิทธิภาพของสารสกัดจากพืชสมุนไพรไทยในการยับยั้งเชื้อยีสต์ที่ขาดยีนซึ่งเกี่ยวข้องกับการควบคุมการดื้อยา

The Antifungal Activity of Thai Herbal Extracts against Yeast Strains with Deletion of Drug Regulatory Genes

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บทคัดย่อ

สารสกัดจากสมุนไพรไทยในรูปแบบของน้ำมันหอมระเหยถูกนำไปใช้อย่างแพร่หลาย เพื่อวัตถุประสงค์ต่างๆ เช่น ใช้ในยาแผนโบราณ แม้ว่าคุณสมบัติด้านการต้านเชื้อราของน้ำมันหอมระเหยได้มีการศึกษาอย่างกว้างขวาง แต่กลไกของการยับยั้งเชื้อรายังไม่เป็นที่แน่ชัด ในการศึกษานี้ได้สกัดน้ำมันหอมระเหยจากกานพลูและอบเชยด้วยตัวทำละลายเฮกเซน แล้วนำไปตรวจสอบคุณสมบัติในการต้านเชื้อราในเชื้อราฉวยโอกาสที่ก่อให้เกิดโรคในมนุษย์ *Candida albicans* และยีสต์ต้นแบบ *Saccharomyces cerevisiae* โดยใช้วิธี spot dilution assays ผลการศึกษาในเชื้อยีสต์ *S. cerevisiae* พบว่าความเข้มข้นต่ำสุดของน้ำมันหอมระเหยที่สามารถยับยั้งการเจริญเติบโตเป็น 250 มิลลิกรัมต่อมิลลิลิตรสำหรับน้ำมันกานพลู และ 100 มิลลิกรัมต่อมิลลิลิตรสำหรับน้ำมันอบเชย ส่วนเชื้อรา *C. albicans* ความเข้มข้นต่ำสุดของน้ำมันกานพลูและอบเชยที่สามารถยับยั้งเชื้อได้คือ 200 และ 100 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ การขาดของยีนที่ควบคุมการถอดรหัส CWT1 มีผลในการเพิ่มความต้านทานของน้ำมันหอมระเหยในเชื้อ C. albicans ดังนั้นน้ำมันกานพลูและอบเชยที่สกัดด้วยเฮกเซนจะมีคุณสมบัติในการต้านเชื้อรา และอาจมีศักยภาพในการพัฒนาเพื่อเป็นยาต้านเชื้อราทางเลือกต่อไป

ABSTRACT

Thai herbal extracts in the form of essential oils are used widely for various purposes such as in traditional medicine. Eventhough the antifungal properties of essential oils have been extensively studied; however, less is known about the mechanisms of inhibition. In this study, a solvent extraction using n-hexane is employed to obtain clove and cinnamon oils for examination of their antifungal activities against the human opportunistic pathogen *Candida albicans* and the model yeast *Saccharomyces cerevisiae*, using spot dilution assays. The results showed that the concentration of herbal oils required for inhibition of growth are 250 mg/ml of clove bud oil and 100 mg/ml of cinnamon bark oil for *S. cerevisiae*. For *C. albicans*, the minimum fungicidal concentration of clove and cinnamon oil was identified to be 200 and 100 mg/ml concentration, respectively. However, deletion of gene, which encodes for a regulatory protein, resulted in increased resistance to both essential oils in *C. albicans*. Thus, clove and cinnamon oil extracted by n-Hexane also has promising antifungal activities and may have potential use in the development of natural products as alternative antifungal agents.

คำสำคัญ: *Saccharomyces cerevisiae*, *Candida albicans*, กิจกรรมในการต้านเชื้อรา, น้ำมันหอมระเหย, กานพลู, อบเชย

Keywords: *Saccharomyces cerevisiae*, *Candida albicans*, antifungal activity, essential oils, clove, cinnamon

INTRODUCTION

Recently, interest in the potential use of natural products to prevent bacterial and fungal growth has sharply increased. Plant products which are generally used for culinary and medicinal purposes are alternative sources of natural compounds. They are often characterized by the various volatile organic compounds that are the secondary products of plant metabolism, which are commonly present in leaves, bark, and fruit (Hili et al., 1997). Steam distillation is one popular method because this process is believed to retain the aromatic healing properties in the most advantageous manner. Solvent extraction is another available extraction method, which is convenient and energy-friendly.

Clove and cinnamon are among the local herbs used in Thailand. Activities of clove bud oil and cinnamon bark oil extracted from steam distillation and the tests against some microbes have been reported (Nejad Ebrahimi et al.; Hili et al., 1997; Prabuseenivasan et al., 2006; Pinto et al., 2009). This includes their antifungal activities against *C. albicans*. This pathogenic fungus resides in the oral cavity and the gastrointestinal tracts and is able to cause several infections, depending on the nature of the host defect (Calderone et al., 2000). *C. albicans* is closely related to the model yeast, *Saccharomyces cerevisiae*. Deletion of *RDS2* gene results in increased sensitivity to the antifungal drug ketoconazole (Akache et al., 2001). *S. cerevisiae* Rds2 has a homologue in *C. albicans* called Cwt1 which is involved in cell wall biogenesis and integrity (MacPherson et al., 2006). Multidrug resistance (MDR) is often caused by overexpression of drug efflux pumps (Moye-Rowley, 2003). This study aimed to characterize the antifungal activities of essential oils obtained from clove bud and cinnamon bark, extracted by n-Hexane, against *C. albicans* and *S. cerevisiae* wild type and deletion strains.

MATERIALS AND METHODS

Essential oils: Two essential oils extracted from clove bud and cinnamon bark were tested. One hundred grams of each dry herb were mashed, dissolved with n-Hexane and extracted by vigorous shaking at RT. The filtrate was collected and, n-Hexane was evaporated (BUCHI Rotavapor R-200).

Microorganisms: The microorganisms used in this study were *C. albicans* SGY243 and Δ*cwt1* (Kelly et al., 1987) and *S. cerevisiae* BY4741 and Δ*rds2* (Brachmann, 1998).

Spot dilution method: The yeast strains were grown in regular liquid yeast peptone dextrose (YPD) overnight. Cells were serially diluted and spotted on appropriated plates, containing 100 μl of herbal oils at the final concentrations of 0 to 250 mg/ml. Cells were incubated at 30 ºC for 48 h. The minimum fungicidal concentration was estimated.

RESULTS AND DISCUSSION

The minimum concentrations of oils required for growth inhibition of yeast strains were determined via the spot dilution assay. For *S. cerevisiae*, the minimum fungicidal concentrations of individual oil were identified to be 250 mg/ml for clove and 100 mg/ml for cinnamon (Figure 1).

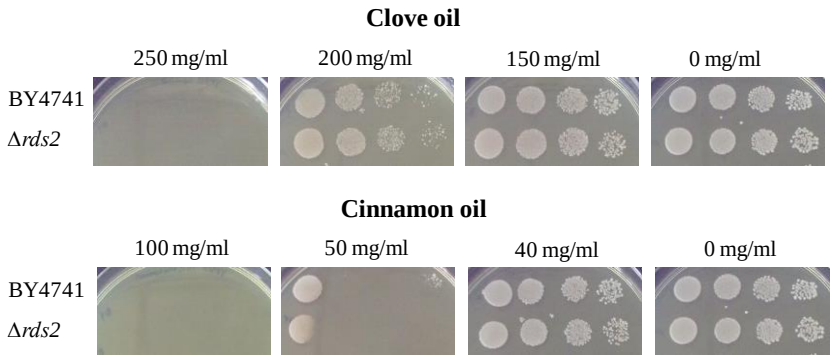

Figure 1 Growth of the *S. cerevisiae* BY4741 and Δ*rds2* strains on plates containing clove bud and cinnamon bark oil in the range of final concentrations of 0-250 mg/ml via the drop diffusion method (see materials and methods for details of the experiment).

In 2008, Moreno and coworkers reported that the lack of Rds2 affects the chemical cell wall compositions of yeast cells. The higher mannan content (1.8-fold) and a lower amount of chitin (0.7-fold) were found in the cell wall of the Δ*rds2* strain as compared to the wild-type strain (Moreno et al., 2008). β-1, 3-glucan that is linked with chitin is an important component of cell wall structure. The lack of chitin induces to cell wall defects. Moreover, other essential oils, such as oregano and clove oils, could also damage the cell surface of *S. cerevisiae* (Chami et al., 2005). Thus, the imperfect cell wall of the Δ*rds2* mutant may allow essential oils to penetrate easily the cell wall and cause damage the cells. Overall, the results suggested that Rds2 plays an important role in the resistance to essential oils in *S. cerevisiae*.

For *C. albicans*, the minimum fungicidal concentrations of clove oil were identified to be 200 mg/ml for the wild-type, and 250 mg/ml for the Δ*cwt1* strain and those of cinnamon oil were approximately 50 mg/ml for the wild-type and 100 mg/ml for the Δ*cwt1* strain (Figure 2). In 2003, Moreno and coworker disrupted the *CWT1* gene from the genome of *C. albicans* SGY243 background and characterized the effect on cell wall composition. They found that the deficiency of Cwt1 leads to a 2-fold increase of mannoproteins (Moreno et al., 2003). Mannoproteins function to fill the gap between the cell wall to protect the cell from environmental stress, thereby preventing essential oils from entering to the cells. Overall, the results suggested that Cwt1 plays a role in mediating resistance/sensitivity to tested essential oils.

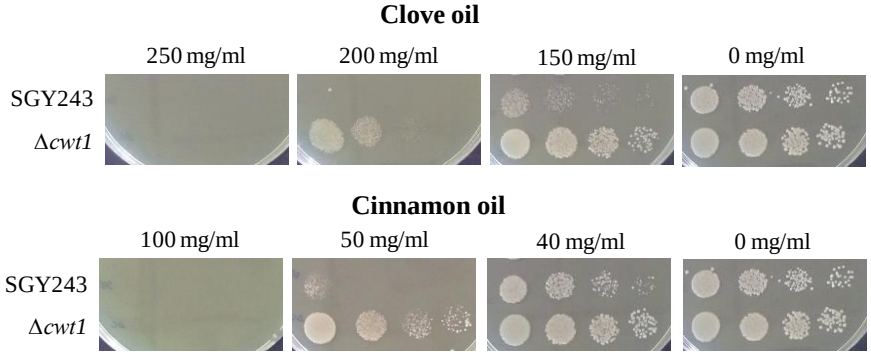

Figure 2 Growth of the *C. albicans* SGY243 and Δ*cwt1* strains on plates containing clove bud and cinnamon bark oil in the range of final concentrations of 0-250 mg/ml via the drop diffusion method (see materials and methods for details of the experiment).

CONCLUSION

In summary, both *S. cerevisiae* Rds2 and *C. albicans* Cwt1 transcription regulators appeared to play an important role in mediating sensitivity to herbal oils, isolated from clove and cinnamon. Our results suggest and support promising antifungal activity of both Thai herbs and showed that the n-Hexane extraction method may also result in the discovery of active ingredients.

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