嘉南藥理科技大學專題研究計畫成果報告

海巴戟天葉粗萃取物之食物機能性研究--

(二)海巴戟天粗萃取物對 *Staphylococus aureus* 的抗菌研究

計畫類別：□個別型計畫　　☑整合型計畫

計畫編號：CNHN93-01

執行期間：93 年 01 月 01 日至 93 年 12 月 31 日

計畫主持人：林翠品 助理教授

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中文摘要

Morinda citrifolia（Rubiaceae）俗名為 Noni，中文名為海巴戟天。海巴戟天果實可以抑制肺癌及肉瘤的生長，也發現果實中的多醣類物質具有免疫調節的功能；以酒精與己烷萃取海巴戟天葉發現具有抗肺結核菌成分。本研究主要是探討海巴戟天對病原菌 *Staphylococcus auerus* 抗菌作用，由於 *Staphylococcus auerus* 是院內最容易感染的細菌，會導致新生兒，傷口，手術後的感染及次級肺炎；而且也發現 *Staphylococcus aureus* 細菌不僅對 b-lactam 抗生素具有抗藥性，也對 macrolides, aminoglycosides， fluoroquinolones 及 vancomycin 具有抗藥性，所以開發天然物並且與抗生素合併使用，是達到抗菌效果的重要方法。本實驗是以熱水、酒精萃取海巴戟天之葉，果實及莖中有效成分，並且評估海巴戟天之各種萃取物對於具有抗藥性 *Staphylococcus aureus* 的抗菌能力以及評估海巴戟天與抗生素抗菌的協同作用。結果顯示以酒精萃取海巴戟天果實對抗藥性 *Staphylococcus aureus* 具有抗菌能力，將海巴戟天與 oxacillin 一起作用發現具有抗菌協同作用。

關鍵詞：海巴戟天，抗菌，*Staphylococcus aureus*。

ABSTRACT

Morinda citrifolia is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, anti-inflammatory, and immune enhancing effects. In this study, the hot water and ethanol crude extracts were isolated from the leaves, fruits, and stems of *Morinda citrifolia*, which were further used to estimate the antibacterial activity against *Staphylococcus aureus*. Our results showed that the ethanol extracts from the fruits of *Morinda citrifolia* reveal the growth inhibition against MSSA and MRSA. Combinations of noni ethanol extracts and oxacillin showed potent synergy against MRSA.

INTRODUCTION

Morinda citrifolia, as known as noni, is a common plant of the Indo-Pacific region and grows through the philipine archipelago. The bark, stem, root, leaf and fruit of the plant have many uses in traditional medicine, including as the treatment for diabetes, hypertension and cancer. Compounds is extracted from *Morinda citrifolia* display various biological activities, such as cardiovascular activity, antitumor activity,

antiviral activity, antimicrobial activity and immunomodulator activity. A crude ethanol extract and hexane fraction show antitubercular activity. Base on these reports which led us screen the extracts isolated from the leaves, fruits, and stems of this plant by using hot water and ethanol against methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA) . Methicillin-resistant *Staphylococcus aureus* has become a major nosocomial pathogen in the past 2 decades. Therapeutic option for MRSA infection are very limited because most MRSA strains are resistant not only to b-lactams but to multiple antimicrobial agents, such as macrolides, aminoglycosides, and fluoroquinolones. Therefore, new chemotherapeutic agents and new approaches are need to combat such multiple-antibiotics-resistant bacteria. Our results showed that the water fraction of ethanol extracts from the fruits of *Morinda citrifolia* reveal the growth inhibition against MSSA and MRSA. Combinations of noni ethanol extracts and oxacillin showed potent synergy against MRSA.

RESULTS

Antimicrobial activity of the hot water extracts

The leaves, brown stems, green stems and fruits crude extracts were isolated by 80 ˚C hot water, and the antimicrobial activity was carried out by colony counting on incubated agar plate. The 25 mg/ml hot water extracts from fruits of *Morinda citrifolia* do not exhibited significant inhibition effect for MRSA and MSSA at 25 mg/ml after 24 hr (Fig. 1 A and B).

Antimicrobial activity of the water and DMSO fraction of ethanol crude extracts

The dried 99.5 % ethanol extracts from noni fruits were dissolved in water and 10 % DMSO. The water soluble fractions have 6 log colony forming units (cfu/ml) of MSSA and MRSA were inactivated by 12 mg/ml and 15 mg/ml, respectively, after 24 hr. The DMSO fractions also have 6 log colony forming units (cfu/ml) of MSSA and MRSA were inhibited by 15 mg/ml (Fig. 2).

Synergy between noni and oxacillin against MSSA and MRSA

3 mg/ml of the water soluble fractions and 5 mg/ml of DMSO fractions reversed the high-level resistance of MSSA and MRSA to oxacillin (Fig. 4).

Reduction of tolerance of MRSA and MSSA to high ionic strength in presence of the DMSO fraction of ethanol extracts from noni fruits.

At 5 mg/ml, 10 mg/ ml and 15 mg/ml largely reduced the tolerance both MRSA and MSSA to high concentrations of NaCl.

DISCUSSION

The water soluble and DMSO fraction of ethanol extracts from the fruits not only exhibited the growth inhibitory effect against MSSA and MRSA, but also reversed the high-level resistance of MRSA to oxacillin. Further studies will investigate the mechanism of growth inhibition and will

screen the antimicrobial activities of ethanol extracts from noni leaves , brown stem and green stem.

EVALUVATION

This research is in line with the progress of grand and achieved to the respect. The results are reported on bacterial conference 2004.

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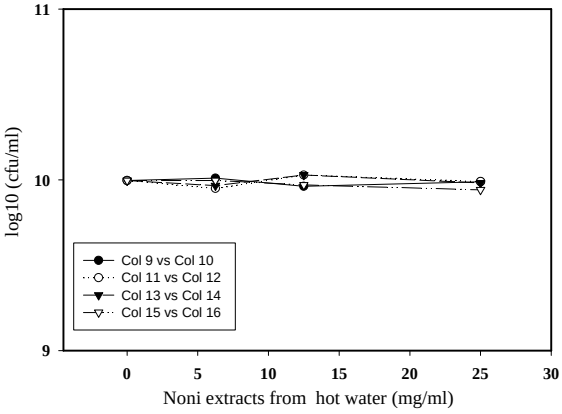
Figures



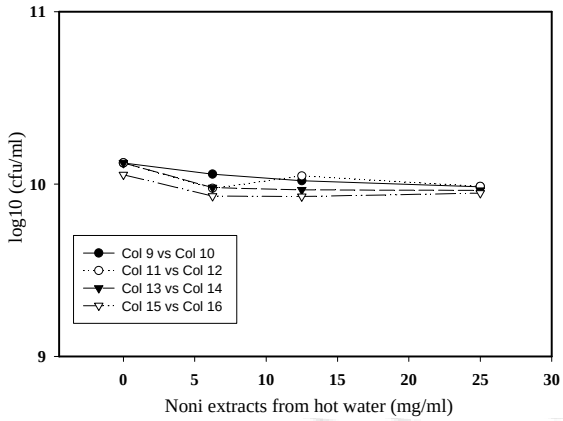


FIG. 1 Antibacterial activities of the hot water extracts against MSSA (A) and MRSA (B). Bacteria were inoculated into 1 ml MHB containing different concentrations of the hot water extracts from the leaves, brown stems, green stems and fruits of noni.

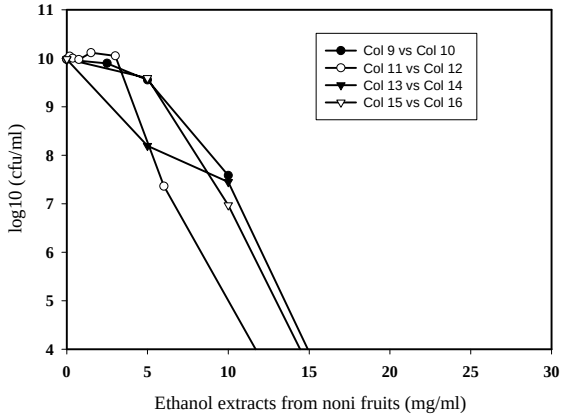
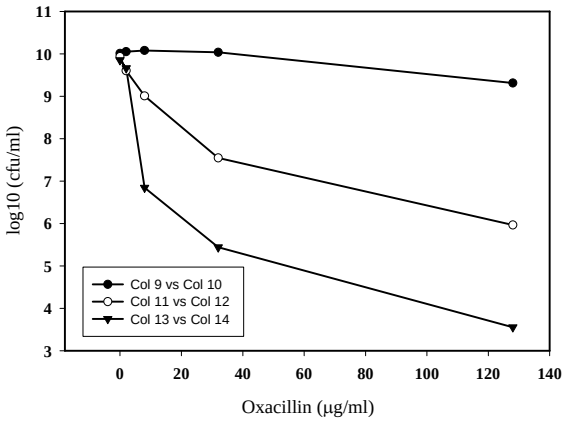
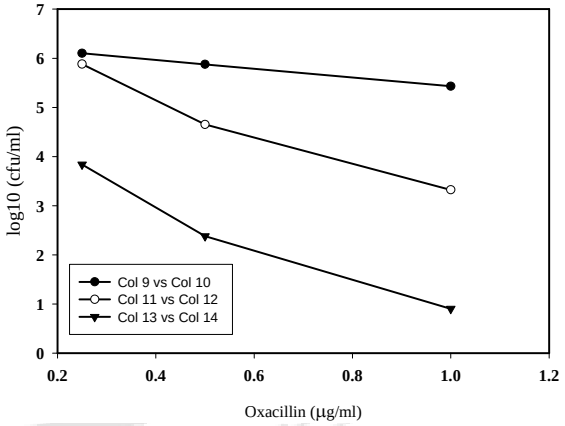


FIG. 2. Antibacterial activities of the water fraction and DMSO fraction of ethanol extracts from noni fruits against MSSA and MRSA.





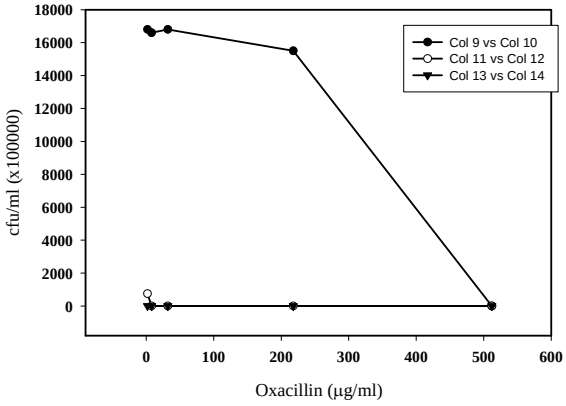
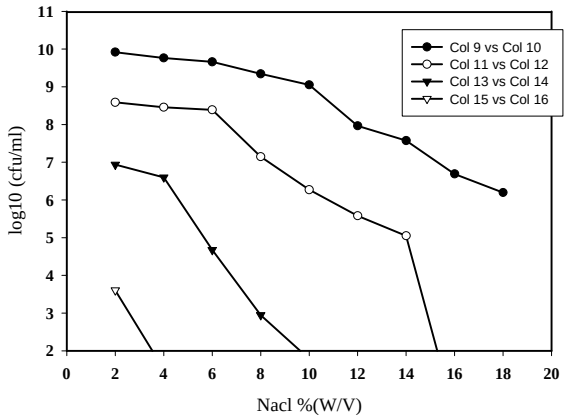


FIG. 3. Synergistic anti-MRSA1and anti-MSSA effects between ethanol extracts of fruits and oxacillin. The ethanol extracts were dissolved in water (A) and 10 % DMSO (B and C) fractions. These solutions were used in the determination of the antibacterial activities against the MRSA (A and C) and MSSA (B)



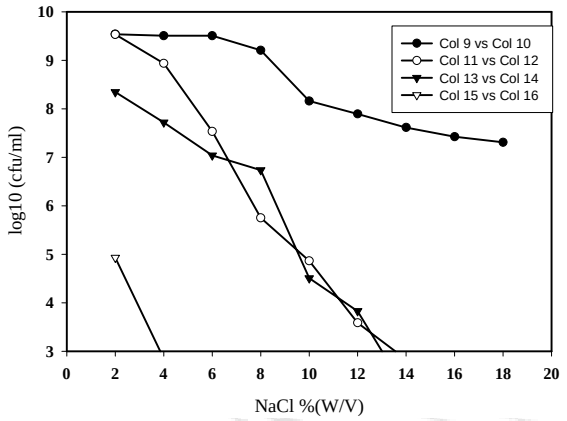


Fig. 4 Reduction of tolerence of MRSA and MSSA to high ionic strength and low osomotic pressure in DMSO fraction of ethanol extracts from noni fruits. (A) MSSA cell (104/ml) were incubated in water with various concentrations of DMSO fractions for 4, 8, 12 and 24 h. MRSA (B) and MSSA cell (106/ml) (C) were cultured in 1 ml of MHB containing different cocentrations of DMSO fraction and NaCl.