

# 嘉南藥理科技大學專題研究計畫成果報告

## 海巴天葉粗萃取物之食物機能性研究

計畫類別：個別型計畫 整合型計畫

計畫編號：CNHN93-01

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子計畫一：以豬肝粒腺體為模式探討海巴天粗萃取物之脂質抗氧化性

計畫主持人：陳師瑩

子計畫二：海巴天粗萃取物對 *Staphylococcus aureus* 的抗菌研究

計畫主持人：林翠品

執行單位：嘉南藥理科技大學

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# 嘉南藥理科技大學補助專題研究計畫成果報告

## 海巴戟天葉粗萃取物之食品機能性研究--

### (一) 以豬肝微粒體為模式探討海巴戟天葉粗萃取物之脂質抗氧化性

計畫類別：個別型計畫      整合型計畫

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#### 一、中英文摘要

海巴戟天(*Morinda citrifolia*)，屬於茜草科(Rubiaceae)，其俗名為Noni，主產於夏威夷群島、大溪地與熱帶亞洲國家，依據民間傳統療法的資料顯示，海巴戟天的樹皮、莖、根、葉、果皆可調製，相傳有糖尿病、高血壓與癌症的預防及治療等醫療保健功效。本研究的目的是評估海巴戟天葉粗萃取物在保護生物細胞膜免於氧化性傷害之能力，本實驗結果顯示海巴戟天葉之絕對酒精粗萃取物對LYD三品系肉豬肝臟微粒體脂質過氧化具有顯著性的抑制能力，此結果可能與其清除氫氧自由基的能力為主有關，並與多酚類物質相關。

**關鍵字：**海巴戟天、脂質過氧化、微粒體。

*Morinda citrifolia*(Rubiaceae), commonly known as Noni, is a plant typically found in the Hawaiian, Tahitian and tropical Asia. The bark, stem, roots, leaves, and fruits have been used traditionally as a folk remedy for many diseases including diabetes, hypertension, and cancer. This study was to prove the leaves of Noni crude extracts in the protection ability of biological cell membrane which were attacked

by oxidative damage. The results indicated that the absolute ethanol extracts of Noni's leaves display significantly the inhibition ability of lipid peroxidation of microsomes from LYD pig liver. The scavenging mechanism of the absolute ethanol extracts of Noni's leaves might have relation with hydroxyl radical. The antioxidant components might be attributed to higher content of polyphenolic compounds.

**Keywords:** *Morinda citrifolia*, Noni, lipid peroxidation, microsomes.

#### 二、緣由、實驗設計與目的

由自由基(Free radical)或活性氧(Reactive oxygen species)所造成的氧化性傷害已成為近年來食品學和生理學研究的重點。醫學上甚至認為許多疾病的發生(如糖尿病、高血壓與癌症等)與老化現象是因自由基或活性氧的產生所造成<sup>(1)</sup>。在我們的飲食當中存在一些具有抗氧化特性的特殊成分，能防止或減緩自由基或活性氧所造成的破壞。*Morinda citrifolia*(Rubiaceae)即是其中之一，其俗名為Noni，中文名為海巴戟天<sup>(2)</sup>，適合生長在本島南部溫溼地帶，氣溫22

°C 以上，台灣的仲夏到季秋為盛產期，由於種植及採收容易，南部農民對於種植該類植物的興趣相當濃厚。如果海巴戟天經由改進調製或萃取的方法，其生物活性研究分析亦得到科學驗證，不僅材料極具本土性開發價值，適合台灣開發量產，也可以帶動南部生物科技產業的發展。近年來對於海巴戟天相關文獻有增加的趨勢，Hirazumi, 於1994<sup>(3)</sup>及1996<sup>(4)</sup>的研究顯示，飲用海巴戟天果實的汁可以促進小鼠免疫系統，並抑制Lewis lung carcinoma 的生長；Liu和Sang等人的研究顯示，海巴戟天葉與果實中含有抑制癌細胞發展的物質<sup>(5,6)</sup>；Hirazumi於1999證實海巴戟天的果實中的多醣類物質 (Polysaccharide-rich substance) 具有免疫調節劑 (immunomodulator) 的生理功能<sup>(7)</sup>；而最近Sang等人<sup>(8)</sup>的研究發現由海巴戟天的葉純化出來的物質 (Flavonol glycosides) 具有抗氧化性；Zin等人於2002年從海巴戟天的葉、根及果實所得到的甲醇與乙酸乙酯萃取得物也分析到抗氧化活性<sup>(9)</sup>。

本實驗室過去的研究顯示<sup>(10-16)</sup>：海巴戟天之葉粗萃取物的體外 (*in vitro*) 試驗，不論是80°C熱水、50% 與99.5% 酒精、乙酸乙酯及超臨界二氧化碳 (SF-CO<sub>2</sub>) 的條件都含有抗氧化特性；然而，各粗萃取物的抗氧化特性卻又不盡相同，因此其抗氧化的作用機制可能也不一致，為了更進一步瞭解海巴戟天葉粗萃取物的抗氧化作用機轉，以及可能接近於體內的抗氧化效應，本實驗擬以豬肝微粒體為模式，優先探討各萃取物對脂質過氧化之保護性分析；由於豬肝取材容易又具有接近人體試驗的代表性，可以免除動物實驗與細胞培養的研究成本與所花費的人力與時間等優點，適合作為評估各類保健食品能否保護脂質過氧化的分析方法；同時，本實驗亦藉此實驗，建立以豬肝微粒體為模式，作為探討保健食品之脂質抗氧化性的標準研究程序。

### 三、實驗材料與方法

研究材料 *Morinda citrifolia* 係購自南部產地，其葉經烘乾後，由磨粉機輾磨裝瓶，並置入除濕器中冷藏備用。萃取方法使用下列四種 (1) 乙酸乙酯萃取 (2) 99.5%EtOH (3) 50%EtOH (4) 80°C 純水。

研究方法係依據 Germano (2002)<sup>(17)</sup> 說明執行。

#### (1) 豬肝微粒體的分離

將3月齡重約30~40 Kg之LYD三品系肉豬解剖取出重約500-600 g之的肝臟，作為測試標的物，以冰冷含1.15% KCl之0.01M KH<sub>2</sub>PO<sub>4</sub> 緩衝液 (pH 7.4, 4°C) 稍沖洗後，拭乾稱重。於4°C下，先以剪刀將其初步剪碎，再將剪碎之肝加入緩衝液20% (w/v) 於離心管中均質。將肝均質液20% (w/v)，於4°C、670 xg 離心10分鐘，將未打破之細胞及細胞核等大分子物質分離。取上層液，再於4°C、100,000xg 離心15分鐘，將粒腺體，溶素體等小分子物質分離。取上層液，於超高速離心(4°C、105,000 xg, 60分鐘)，得沉澱物微粒體。將所得沉澱微粒體加入1~2 mL的緩衝液使成微粒體懸浮液。將微粒體懸浮液置於90°C加熱1.5分鐘以去除反應時的酵素影響，並將所得的微粒體懸浮液進行蛋白質定量。

#### (2) 脂質過氧化作用之測定

總體積1ml反應液，包括測試樣品，微粒體懸浮液(0.5mg protein/mL)，FeSO<sub>4</sub> (終濃度為10 μM) 和 ascorbic acid (終濃度為0.1 mM) 以引發過氧化反應。在37°C水浴中振盪 (180 rpm) 反應一小時，將上述反應液取出0.5 ml，加入1 ml TCA-TBA-HCl 混合液 (15% w/v TCA, 0.375% w/v TBA, 0.25N HCl)，均勻混和後，加蓋，在沸水浴中加熱15分鐘。於冷水中冷卻至室溫，以8,000 xg，離心10分鐘，取上清液在波長532nm下測定其吸光值。

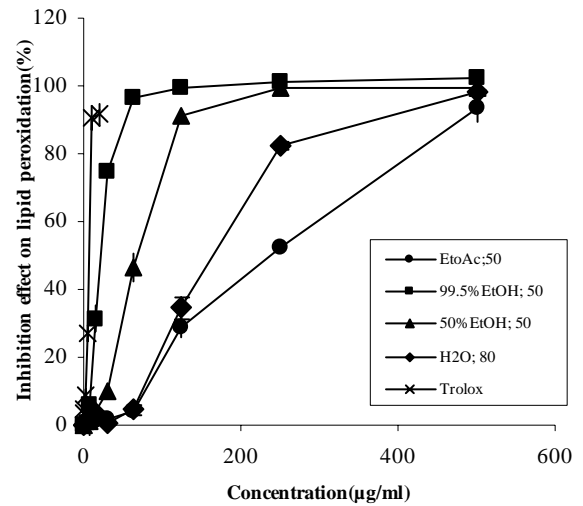
#### (3) 統計分析

本研究之分析項目皆進行三重複之測定，所得實驗數據以 SAS/PC 統計分析軟體進行分析，以 ANOVA 程序做變異數分析並以 Duncan's multiple range tests 做顯著性差異比較。

#### 四、結果與討論

海巴戟天葉四種溶劑萃取物對於豬肝微粒體脂質過氧化的抑制情形如圖一所示，隨著樣品濃度的上升，抑制脂質過氧化的能力亦有隨之上升的趨勢，在樣品濃度為 125  $\mu\text{g}/\text{mL}$  時，葉 99.5% 乙醇及 50% 乙醇萃取物的抑制效果均達到 90% 以上，而純水及乙酸乙酯萃取物則較差，分別為 34.7% 及 29.0%。因此顯示海巴戟天葉之乙醇萃取物具有較佳抑制脂質過氧化能力。若計算出各樣品抑制 50% 微粒體脂質過氧化所需的濃度 ( $\text{EC}_{50}$ )，正標準品 Trolox 為  $6.81 \pm 0.54 \mu\text{g}/\text{mL}$ ，而葉 99.5% 乙醇及 50% 乙醇萃取物分別為  $28.55 \pm 6.83$  及  $90.09 \pm 0.95 \mu\text{g}/\text{mL}$ ，亦即二者抑制微粒體脂質過氧化的  $\text{EC}_{50}$  值分別約為 Trolox 之 4 倍和 13 倍如表一所示。

依據過去的研究顯示<sup>(10-16)</sup>海巴戟天葉之乙醇粗萃取物在不同溶劑萃取下，存在各式抗氧化活性的能力，顯示海巴戟天的葉中存在極性與非極性的抗氧化成分；高螯合鐵能力成分傾向存在於葉中極性部分，清除  $\text{O}_2^{\cdot -}$  與  $\text{H}_2\text{O}_2$  的能力傾向存在於葉中非極性部分，清除氫氧自由基能力則多存在於葉中醇溶性部分；並且海巴戟天葉之乙醇粗萃取物具有較高的總酚類化合物及類黃酮含量，可能是參與其抗氧化活性之作用的主要成份。因此，推測海巴戟天葉能得到較高抗氧化活性可能與總酚類化合物及類黃酮之化學成份有關。



圖一、海巴戟天葉的不同溶劑萃取物對豬肝微粒體脂質過氧化之保護作用

Figure 1. Effect of crude Noni's leaves extracts by various solvent extractions on lipid peroxidation from pig liver microsome. All samples were tested at five to eight concentrations and each value was means of three replicate analysis.

表一、海巴戟天葉的不同溶劑萃取物抑制豬肝微粒體脂質過氧化之  $\text{EC}_{50}$

Table 1.  $\text{EC}_{50}$  of crude Noni's leaves extracts by various solvent extractions on lipid peroxidation from pig liver microsome.

Sample	$\text{EC}_{50}$ ( $\mu\text{g}/\text{ml}$ )
Noni's leaves (EtOAc ; 50 )	$226.00 \pm 13.93^e$
Noni's leaves (99.5% EtOH ; 50 )	$28.55 \pm 6.83^b$
Noni's leaves (50% EtOH ; 50 )	$90.09 \pm 0.95^c$
Noni's leaves ( $\text{H}_2\text{O}$ ; 80 )	$164.77 \pm 4.42^d$
Trolox	$6.81 \pm 0.54^a$

1. Each value is the mean  $\pm$  SD ( $n=3$ ).

2.  $\text{EC}_{50}$  : the efficient concentration of crude Noni extracts caused 50% inhibition effects on lipid peroxidation.

## 五、計劃成果總結

1. 本實驗可以進一步瞭解海巴戟天葉粗萃取物的抗氧化作用機轉，以及可能接近於體內的抗氧化效應。
2. 本實驗建立以豬肝微粒體為模式，探討各萃取物對脂質過氧化之保護性分析，由於實驗與操作過程已趨於穩定，以及容易操作，可作為探討保健食品之脂質抗氧化性的標準研究程序。
3. 本實驗結果顯示海巴戟天葉之絕對酒精粗萃取物對 LYD 三品系肉豬肝臟微粒體脂質過氧化具有顯著性的抑制能力，此結果可能與其清除氫氧自由基的能力為主有關，並與多酚類物質相關。

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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 *Staphylococcus aureus* 的抗菌研究

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計畫主持人：林翠品 助理教授

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執行單位：嘉南藥理科技大學 保健營養系

中華民國九十四年二月二十八日

# 嘉南藥理科技大學專題研究計畫成果報告

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主持人：林翠品 嘉南藥理科技大學 保健營養系

## 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 aureus* 抗菌作用，由於 *Staphylococcus aureus* 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. Based on these reports which led us to 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 options for MRSA infection are very limited because most MRSA strains are resistant not only to  $\beta$ -lactams but to multiple antimicrobial agents, such as macrolides, aminoglycosides, and fluoroquinolones. Therefore, new chemotherapeutic agents and new approaches are needed 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 exhibit 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.

## EVALUATION

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

## REFERENCE

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Figures

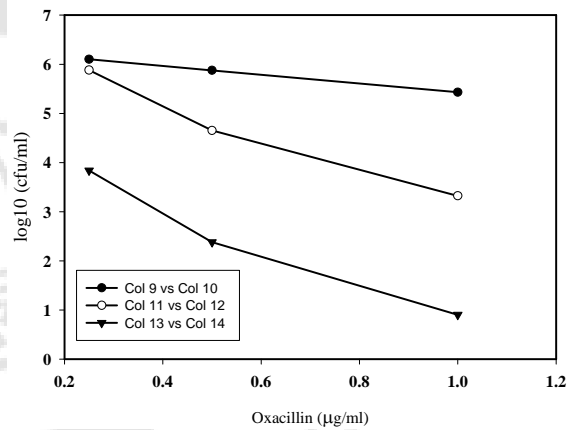
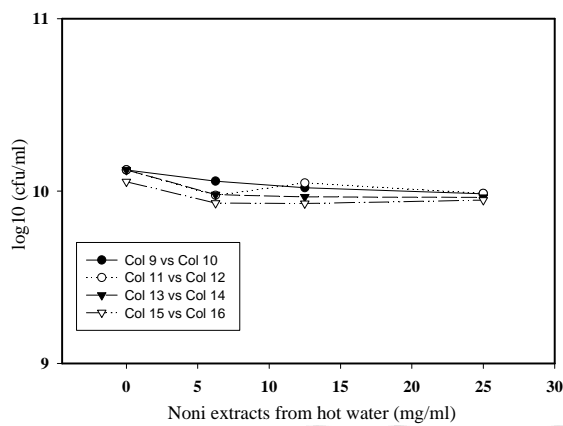
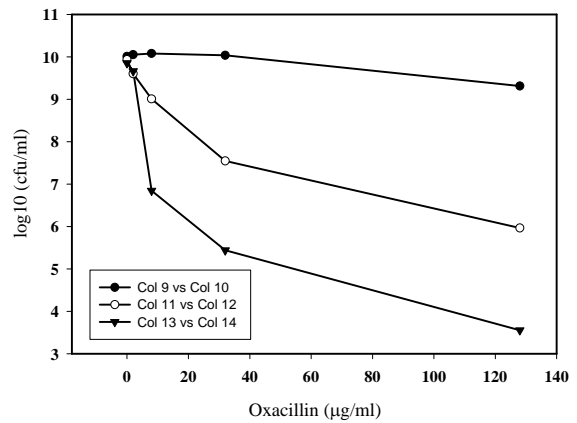
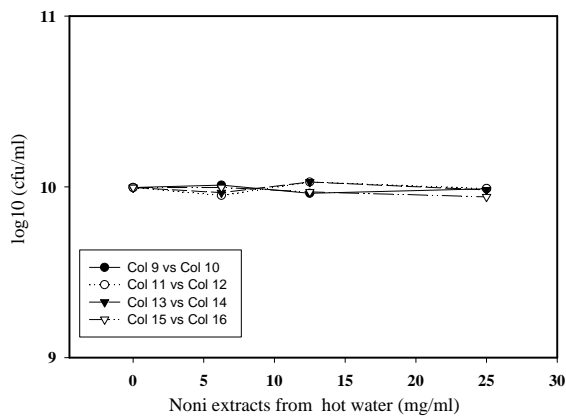


FIG. 1 Antibacterial activities of the hot water extracts against MSSA (A) and MRSA (B). Bacteria were inoculated into 1 ml MHB containing different concentrations of the hot water extracts from the leaves, brown stems, green stems and fruits of noni.

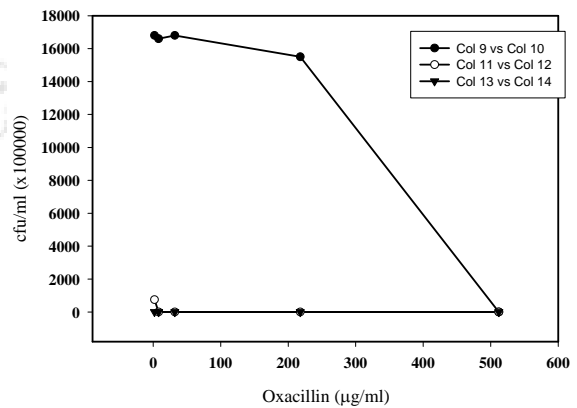
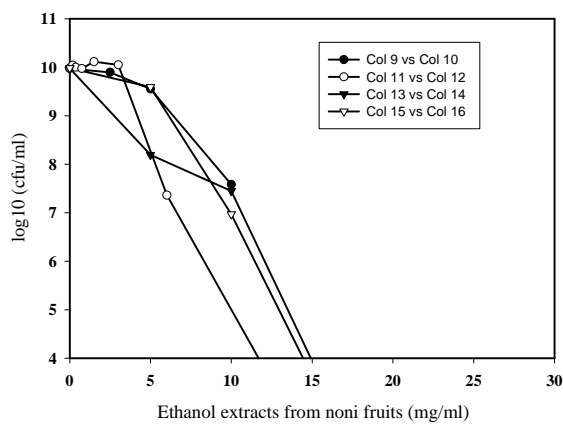


FIG. 2. Antibacterial activities of the water fraction and DMSO fraction of ethanol extracts from noni fruits against MSSA and MRSA.

FIG. 3. Synergistic anti-MRSA and 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)

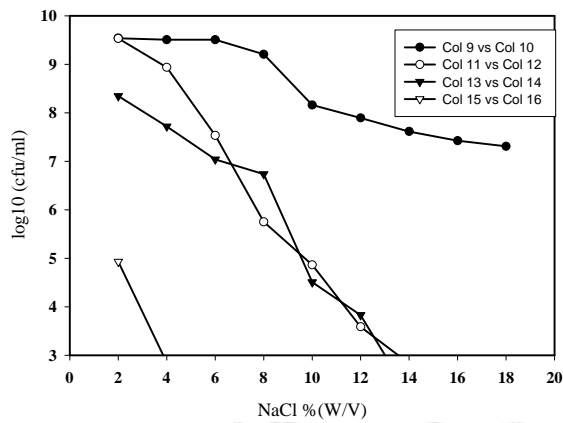
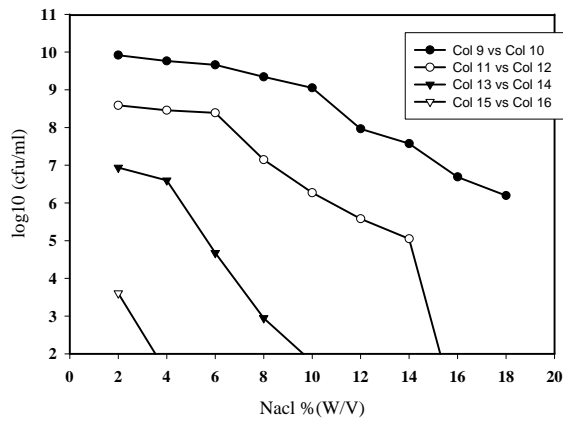


Fig. 4 Reduction of tolerance of MRSA and MSSA to high ionic strength and low osmotic pressure in DMSO fraction of ethanol extracts from noni fruits. (A) MSSA cell (104/ml) were incubated in water with various concentrations of DMSO fractions for 4, 8, 12 and 24 h. MRSA (B) and MSSA cell (106/ml) (C)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