

**Antimicrobial and antiproliferative property of *Azadirachta indica* A.Juss (Neem).**

Amal Kumar Ghimeray, Eom Seok Hyun, Jin-Cheng-Wu, Bimal Kumar Ghimire, Park Hyoung Jae, Kim Won Woo, Kim Seul Gi, Cho Dong Ha\*  
School of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, S.Korea.

***Azadirachta indica* A.Juss (Neem)의 항균 및 항증식성 기능**

강원대학교 : Amal Kumar Ghimeray, 엄석현, 김성무, Bimal Kumar Ghimire, 박형재, 김원우, 김슬기, 조동하\*

**Objectives**

*Azadirachta indica* A. Juss, commonly called as Neem, (family Meliaceae) has been widely used for medicinal purposes. The objective of this experiment was to compare the neem leaf and bark antimicrobial activity on food borne pathogens using different solvent extracts and to evaluate the antiproliferative property against HT-29 colon cancer cells and NCI-N87 human gastric cancer cell line.

**Materials and Methods**

Antimicrobial activity of the Neem leaf and bark was determined according to the disc diffusion method (Lenette et al 1985).

Antiproliferative property of neem leaf and bark was assessed by MTT assay according to Mosmann et al 1983. briefly, HT-29 colon cancer cells and NCI-N87 human gastric cancer cell line were plated in 96-well plates in 200 $\mu$ g/ml of culture medium for 24 h, then exposed to extract with a series of concentration (62.5, 125, 250, 500, 1000  $\mu$ g/ml) for 24, 48 and 72 h time intervals. 20  $\mu$ l MTT solution (2 mg/mL in PBS) was added to each well from incubation with the extract for 24, 48 and 72h. After 4 h incubation, the supernatant was discarded and 200  $\mu$ l DMSO was added to each well to terminate the reaction. The absorbance was measured at 550 nm using an ELISA plate reader.

**Results**

The antimicrobial effect of Neem leaf and bark on different pathogens revealed that the bark has strong effect in most of the strain used compared to that of Neem leaf (Table 1 and 2).

The MTT assay results showed that the Neem bark butanol and water solvent fraction has strong antiproliferative effect than that of Neem leaf extracts used against HT-29 colon cancer cells and NCI-N87 human gastric cancer cell line

---

Corresponding author : Dong Ha Cho E-mail :chodh@kangwon.ac.kr Tel : 033-250-6475

(Fig 1a,b; 2a,b; 3a,b and 4a,b). These results further proof the strong pharmaceutical property of Neem plant.

\* 시험성적

Table 1. Inhibitory response of Neem bark extracts on test microbes.

Solvent extract used <sup>o</sup>	Inhibition zone(mm) <sup>o</sup>								
	Ba <sup>o</sup>	Sf <sup>o</sup>	Kp <sup>o</sup>	Pv <sup>o</sup>	Lp <sup>o</sup>	Pa <sup>o</sup>	Sa <sup>o</sup>	Pm <sup>o</sup>	Ca <sup>o</sup>
80%MeoH <sup>o</sup>	* <sup>o</sup>	7 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	7 <sup>o</sup>
80%EtoH <sup>o</sup>	5 <sup>o</sup>	* <sup>o</sup>	7 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	8 <sup>o</sup>
Hexane f. <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	12 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>
Ethyl acet f. <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	5 <sup>o</sup>	* <sup>o</sup>	7 <sup>o</sup>	4 <sup>o</sup>	* <sup>o</sup>
Butanol f. <sup>o</sup>	4 <sup>o</sup>	5 <sup>o</sup>	* <sup>o</sup>	8 <sup>o</sup>	* <sup>o</sup>	5 <sup>o</sup>	6 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>
water f. <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	4 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	6 <sup>o</sup>	7 <sup>o</sup>
Tetracycline (control) <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	- <sup>o</sup>
mycostantin (control) <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	+ <sup>o</sup>

Bacillus cereus (Bc), Shigilla fleneri (Sf), Klebsiella pneumoniae (Kp), Proteus vulgaris (ps), Lactobacillus plantarum (Lp), Pseudomonas aruginosa (Pa), Staphylococcus aureus (Sa), Proteus vulgare (Pv), Candida albicans(Ca). \*not detected, "+" detected, "-" not done.

Table 2. Inhibitory response of Neem Leaf extracts on test microbes.

Solvent extract used <sup>o</sup>	Inhibition zone(mm) <sup>o</sup>								
	Ba <sup>o</sup>	Sf <sup>o</sup>	Kp <sup>o</sup>	Pv <sup>o</sup>	Lp <sup>o</sup>	Pa <sup>o</sup>	Sa <sup>o</sup>	Pm <sup>o</sup>	Ca <sup>o</sup>
80%MeoH <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	7 <sup>o</sup>
80%EtoH <sup>o</sup>	4 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	7 <sup>o</sup>
Hexane f. <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	11 <sup>o</sup>	5 <sup>o</sup>	* <sup>o</sup>
Ethyl acet f. <sup>o</sup>	6 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	4 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>
Butanol f. <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	5 <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>
water f. <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	* <sup>o</sup>	8 <sup>o</sup>
Tetracycline (control) <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	+ <sup>o</sup>	- <sup>o</sup>
mycostantin (control) <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	- <sup>o</sup>	+ <sup>o</sup>

Bacillus cereus(Bc), Shigilla fleneri (Sf), Klebsiella pneumoniae (Kp), Proteus vulgaris (ps), Lactobacillus plantarum (Lp), Pseudomonas aruginosa(Pa), Staphylococcus aureus(Sa), Proteus vulgare (Pv), Candida albicans(Ca). \*not detected, "+" detected, "-" not done.

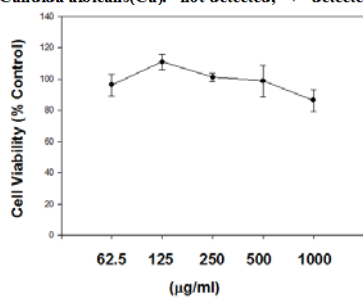


Fig 1a. Effect of Neem leaf water fraction solvent extract on HT-29 Cell.

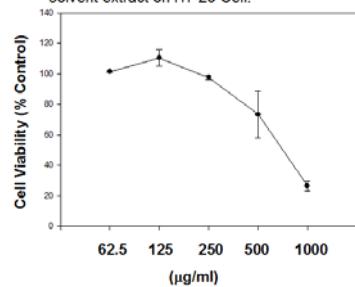


Fig2a. Effect of Neem bark Butanol fraction solvent extract on HT-29 cell.

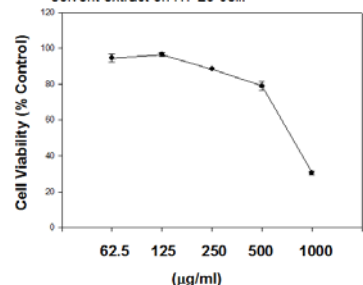


Fig3a. Effect of Neem leaf water fraction solvent extract on NCL-N87cell.

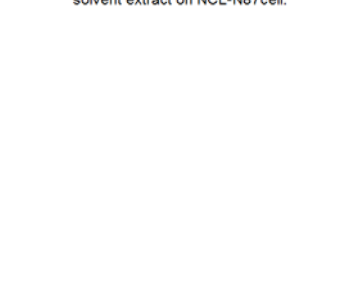


Fig4a. Effect of Neem bark water fraction solvent extract on NCL-N87cell.

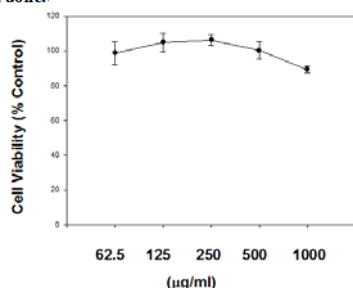


Fig1b. Effect of Neem leaf Butanol fraction solvent extract on HT-29 cell.

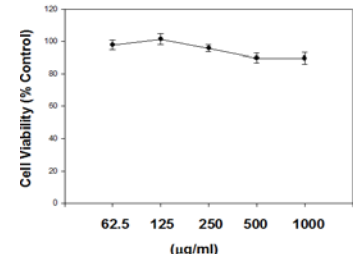


Fig3b. Effect of Neem leaf Butanol fraction solvent extract on NCL-N87 cell.

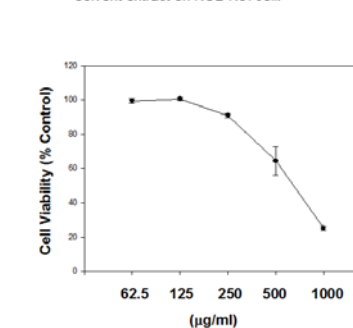


Fig2b. Effect of Neem bark Butanol fraction solvent extract on HT-29 cell.

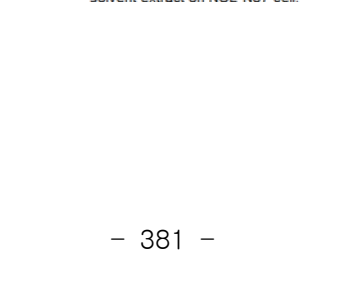


Fig4b. Effect of Neem bark Butanol fraction solvent extract on NCL-N87 cell.