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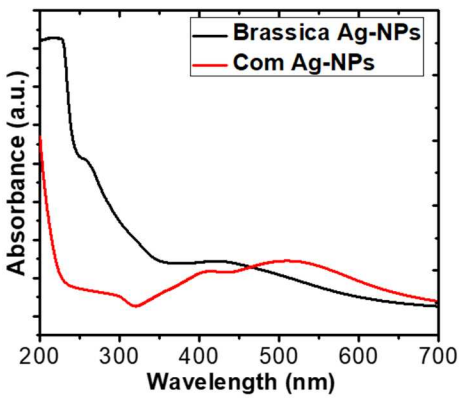
Antibacterial activity and cytotoxicity *in vitro* of green-synthesized silver nanoparticles using *Brassica rapa* var. *japonica* leaf

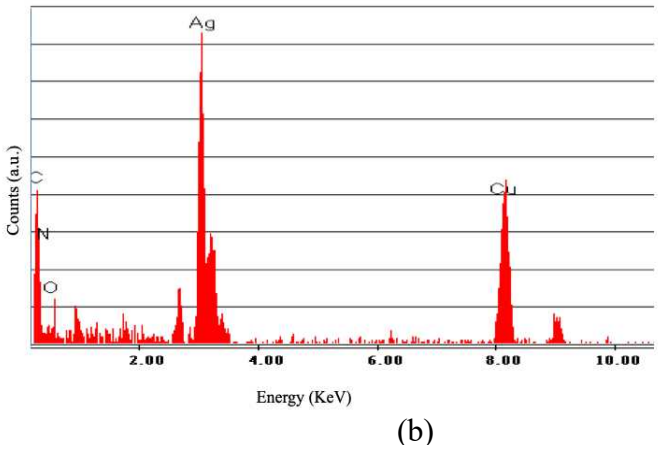
(ミズナの葉を用いてグリーン合成された銀ナノ粒子の
抗菌活性および試験管内細胞毒性)

Abstract

In recent era versatile applications of silver nanoparticles (Ag-NPs) have been elevated by various requirements from the consumers and researchers. According to these requirements, tremendous amounts of Ag-NPs have been synthesized using conventional method. Unfortunately, conventional method for synthesis of Ag-NP has been pointed out a matter of concern in respect of environmental toxicity and human health. Already cytotoxic effects of Ag-NPs have been reported in several cell lines. Therefore, the green synthesis of Ag-NPs is considered to be a safer synthesis method, even though it is an alternative to the conventional synthesis method. On the other hand, Ag-NPs are reported to have potential antitumor and anticancer properties in both *in vitro* and *in vivo* experiments. From above viewpoints, the present study aimed to be green synthesis of Ag-NPs and evaluation their biomedical applications with underlying mechanisms. To achieve the purpose actual objectives were set. First, Ag-NPs were successfully synthesized from the reduction of Ag^{+} using $AgNO_3$ solution as a precursor and *Brassica rapa var. nipposinica/japonica* leaf extract as a reducing and capping agent. In the synthesis procedure no additional chemical reductant and stabilizing agents were used. The characterization of Ag-NPs was carried out using UV-vis spectrometry, energy dispersive X-ray (EDX) spectrometry, fourier transform infrared (FT-IR) spectrometry, field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), atomic absorption spectrometry (AAS), and transmission electron microscopy (TEM).



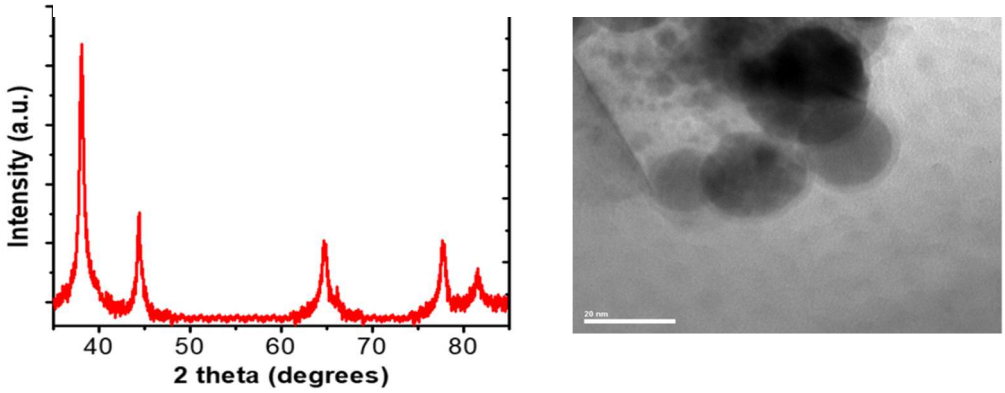
(a)



(b)

UV-vis spectrometry, EDX, XED and TEM are shown in fig 1 and 2.

Fig.1 (a) UV. Vis spectroscopy and (b)EDX of Brassica Ag-NPs



(a) (b)

Fig.2 (a) XRD pattern of Brassica Ag-NPs demonstrating crystalline phase of Ag-NPs (b) TEM images of a Brassica Ag-NPs showing the spherical shaped NPs with the size of 15 to 30 nm

The analyses data revealed the successful synthesis of nano-crystalline Ag possessing high stability than commercial Ag-NPs. To confirm synthesis of Ag-NPs exhibiting less toxicity with high antibacterial activity, following experiments have been done. The cytotoxicity of Brassica Ag-NPs was compared with commercial Ag-NPs using PC12 cell system. 3µg/mL of commercial Ag-NPs reduced cell viability to 23% (control 97%) (fig. 3) and increased lactate dehydrogenase activity, whereas, Brassica Ag-NPs did not show any cytotoxicity on both parameters in PC12 cells. Moreover, Brassica Ag-NPs exhibited zone of inhibition against *Escherichia coli* (11.1 ± 0.5 mm) and *Enterobacter* sp. (15 ± 0.5 mm) (fig. 4) which was higher than other green-synthesized Ag-NPs reported previously.

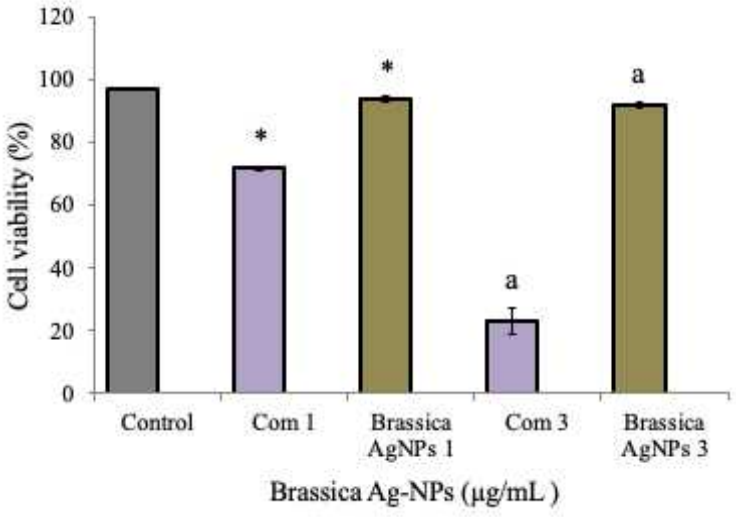


Fig. 3 Cell viability of PC12 cells measured by trypan blue staining method with/without treated by Com Ag-NPs and Brassica Ag-NPs in two different concentrations (1 and 3 µg/mL) for 24 h of incubation. Control group contains cells with medium. Error bars indicate mean± S.E.M (n=3). Asterisk* and a denote significant change from com Ag-NPs to Brassica Ag-NPs at $p<0.05$

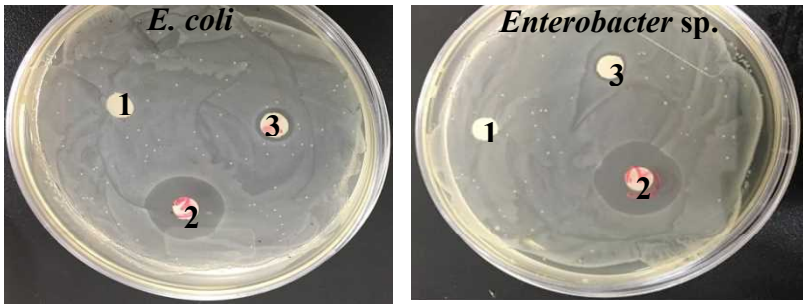


Fig. 4 Antibacterial activity of Brassica Ag-NPs against *E. coli* (a) and Enterobacter sp. (b) in terms of zone of inhibition at a concentration of 10 µg/mL. 1, 2 and 3 denote blank, Brassica Ag-NPs and amphicillin respectively. Amphicillin represents positive control and blank represents negative control.

The less cytotoxicity and high antibacterial activity of green synthesized Ag-NPs will be great benefits for the safe use of Ag-NPs in consumer products. On the basis of results in this study it could be concluded that cytotoxicity of Ag-NPs is depended on the stability of the particles and the stability depends on the encapsulation or coating of the surface of the particles. Therefore, it was considered that reaction temperature during synthesis could play a vital role in coating of the particles. From the results, it was tried to synthesize optimal Ag-NPs using *Brassica rapa var. nipposinika/japonica* leaf extract with various temperatures. The synthesis of Ag-NPs was done at four different temperatures such as 25 °C (room temperature), 60 °C, 80 °C and 100 °C in order to evaluate the extent of encapsulation of Ag-NPs. The synthesized Ag-NPs were again characterized using UV-vis. spectrophotometer, EDX spectrometer, XRD spectrometer, TEM, and dynamic light scattering techniques. The adopted characterization techniques clearly demonstrate that at 100 °C almost all particles were found to be encapsulated which was the primary objective of the present study.

Furthermore, in this study, the behavior of various concentrations of green synthesized Ag-NPs in cancer cells was clarified. Brassica Ag-NPs exposed to Caco-2 cells showed

significant decrease of the cell viability, increase of the LDH activity in the medium, and decrease of intracellular GSH amounts. Subsequent western blotting analyses revealed that Brassica Ag-NPs induced Beclin 1 mediated autophagic cell death in Caco-2 cells where LC3-II plays a key role. This autophagic process was further accelerated via upregulation of p53. Hence, downregulation of Akt suppressed mTOR activation. Moreover, upregulation of IkB and downregulation of NFkB inhibit DNA transcription which might also promote autophagy and subsequent cell death. Involvement of apoptosis or necrosis behind cell death mechanism in Caco-2 cells was not detected from any of the results in current study. Thus, these results indicated the possibility of anticancer ability of Brassica Ag-NPs to Colorectal cancer cells, Caco-2.

In conclusion, this study clearly reveals the potentiality of Brassica leaf extract for the environment friendly green synthesis of Ag-NPs which can be encapsulated with optimal temperature. In addition, Brassica Ag-NPs are less toxic in comparison of commercial Ag-NPs with high antibacterial activity. Moreover, Brassica Ag-NPs induced autophagy regulated type II cell death in colorectal cancer cell which might be a new insight for the therapeutic agent of colorectal cancer.