centrifugation. Purified phage was named as EF1.

Transmission electronmicroscopy (TEM): Purified phage solution (8 μl) was dropped onto 400-mesh Formvar carbon-coated copper grids. After 2 min, the grids werestained with 8 μl of 2% aqueous uranyl acetate and examined with a Philips TECNAI F12 FEI transmission electron microscope at an accelerating voltage of 120 kV.

Phage adsorption and growth curve: An overnight culture of *C. freundii* was inoculated into fresh medium and incubated with shaking at 37℃ for approximately 2 h. Phage EF1 at an approximate MOI of 10 was added to this culture. Samples were periodically withdrawn and immediately chilled while being further diluted to measure total phage activity by the double-layered-agar plate technique.

Antimicrobial activity at different temperatures and pH: The pH of the nutrient broth was adjusted with either 1 M HCl or 1 M NaOH to obtain a pH within the range of 2–11. A total of 100 μl of bacteriophage suspension was inoculated into 10 ml of pH-adjusted medium. After incubation for 30 min at 37℃, the surviving phages were diluted and counted immediately using the softagar overlay method at 37℃. Moreover, the stability of EF1 at various temperatures (40℃, 50℃, 60℃, 70℃, and 80℃) was checked by incubating the phage at the indicated temperature for 30 min at pH 7.0 in nutrient broth; the surviving phages were then counted using the soft agar overlay method at 37℃.

Results: TEM-based showed EF1 has an icosahedral capsid and long contractile tail. The phage head was 103.51 ± 1.25 nm in length and 94.45 ± 1.85 nm in width, while its tail was 66.01 ± 1.49nm in length. Based on these morphological characteristics, EF1 belongs to yoviridae family. Phage EF1 and 3 of its natural hosts, *Citrobacter* isolates *C.freundii*, *C. braakii*, and *C. murliniae*, are described in this study. The 3 strains have similar sensitivities to EF1. Several aspects of the life cycle of EF1 were investigated using the most sensitive isolate, *C. freundii*, under optimal growth conditions. EF1 infects *C. freundii* with moderate latent period, approximately 25 min, and large burst size averaged $5X10^9$ per infected cell. Moreover, the antimicrobial activity of EF1 was well maintained at diverse conditions such as hightemperatures between 40℃, and at 50℃ and wide pH levels ranging from 4 to 11.

Conclusions: This study provides information about a novel virulent *C.freundii* phage EF1. This phage has a high pH stability and high heat resistance. These characteristics increase the utility of this phage as an antibacterial agent. Our future research will examine the application of this characterized phage in treating infections by *C.freundii*.

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Neuroprotective effect of *Sigesbeckia pubescens* extract onglutamate-induced oxidative stress in HT22 cells via downregulation of MAPKs and caspase pathway

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Introduction: Recently, attention has been concentrated onnatural medicine with neuroprotective activities that scavenge free radical andprotect cells from oxidative damage. *Sigesbeckia pubescens* (SP), a traditional Chinese medicinal plant and itsextracts have antioxidant and anti-inflammatory activities. To evaluate theneuroprotective activity of SP extract on glutamate-induced oxidative stress in mousehippocampal HT22 cells.

Materials and Methods: Neuroprotectiveeffects of SP on glutamate-induced oxidative stress in HT22 cells were studiedby analyzing the cell viability by MTT assay, determination of intracellularROS generation and LDH release by the commercial kit, qPCR and Western blotanalysis for observing alterations of neuronal cell death by related pathways.

Results: Pretreatmentwith SP resulted in suppression ofcytotoxicity, ROS generation and LDH release in HT22 cells. To elucidate thepossible molecular pathways of neuroprotection by SP we explore the activationof mitogen-activated protein kinases (MAPKs). Glutamate elicited the mRNAexpression levels of MAPKs (ERK, JNK and p38), whereas notable inhibition ofthese alterations in mRNA levels after co-treatment with SP. Moreover, SP downregulates the expression of MAPKs and caspase3 proteins.

Conclusions: Ourstudy suggests that SP may neuroprotective effects via both alteration of MAPKsand caspase3 pathway under oxidative stress and has potentials agents forpreventing the oxidative neuronal death.

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Hydrangea macrophylla methanolic extract attenuates sodiumarsenite- induced cytotoxicity in HepG2 cells

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Introduction: *Hydrangea macrophylla* (HM) a memberof Saxifragaceae family, is widely cultivated and used as traditional medicinesin China, Japan and Korea. Ithas been revealed to have antioxidant activity. Environmental