

# Ginger Extract Inhibits the Replication of T2 Bacteriophage by Inhibiting the Synthesis of Nucleosides

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## Abstract

Extracts of ginger inhibit the growth of bacteria and viruses of animal cells. At concentrations of 1, 2, 4, and 6 ml of a ginger extract per 100 ml of LB broth, *E. coli* replication was 104.6, 117.7, 117.7, and 122.8% of the control. At concentrations of 2, 4, and 6 ml of ginger extract per 100 ml of LB broth, the yield of T2 bacteriophage decreased by 36.2, 41.8, and 43.7%, respectively. The longer ginger extract was in contact with the bacterial cells prior to infection, the larger was the inhibition of T2 bacteriophage yield. When added 50 min before infection, the yield of T2 bacteriophage was 93.7% of the control, at 100 min prior to infection, the yield was 87.0% of the control and at 150 min prior to infection the yield was 79.9% of the control. The addition of excess glutamine partially reversed the inhibition of T2 yield by ginger extract. With 2 ml of ginger extract, the yield of T2 phage was 71.3% of the control. With ginger extract and 30 mM glutamine, the yield was 81.4% of the control and with 45 mM glutamine, the yield was 93.0% of the control. With ginger extract plus 1, 3 and 5 mM nucleosides, the yield was 129.85, 206.1 and 203.0% of the control. These results suggest that one or more chemicals in ginger extract inhibit the replication of T2 bacteriophage by inhibiting the metabolism of glutamine and the synthesis of nucleotides.

**Keywords:** Ginger extract, T2 bacteriophage, glutamine, nucleosides

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## INTRODUCTION

Ginger is a well-known spice produced from the rhizome, the underground stem, of the tropical herbaceous plant, *Zingiber officinale* and has been used as a food spice and as a medicinal preparation for thousands of years [1]. Extracts of ginger have been successfully tested for treatment of nausea and vomiting during pregnancy [2], as an anti-inflammatory treatment [3], as a treatment for diabetes [4], and as a treatment for certain cancers [5, 6].

Extracts of ginger have been shown to have antibacterial activities. Ginger extracts inhibit the growth of multiple drug resistant strains of *E. coli*, *Pseudomonas aeruginosa*, *Proteus*, and *Staphylococcus aureus* [7] and strains of Gram-negative bacteria that cause periodontal disease [8]. Among the several chemicals identified in ginger extracts [9], at least four different chemicals have been determined to exhibit antimicrobial activity including gingerol [8], paradol [10], zingerone [11], and zerumbone [12]. Ginger extracts have also been shown to have antiviral activities. Ginger

extracts inhibit the replication of Herpes simplex virus type I [13] and respiratory syncytial virus [14]. In this study, the inhibitory effects of a ginger extract on the replication of T2 bacteriophage in *E. coli* were studied.

## MATERIALS AND METHODS

### Ginger Extract Preparation

1.5 gm of ginger root (BulkSupplements.com) was vigorously ground with a glass mortar and pestle in 3 ml of ethanol and 3 ml of Luria-Bertani (LB) broth. 44 ml of LB broth was added and the suspension was centrifuged to sediment the undissolved particles. The supernatant liquid was filter sterilized, stored at 4°C and used for experimental purposes within 24 h.

### Bacterial Cells

Stock cultures of *E. coli* ATCC strain 25250 were prepared on nutrient agar slants incubated at 37°C for 24 h and were stored at 4°C. LB broth cultures were prepared by inoculating 50 ml of sterile LB broth in 125 ml

Erlenmeyer flasks with a loop full of stock bacteria and incubated overnight on a rotary incubator set at 37°C with constant agitation at 125 rpm.

### **Bacteriophage**

A stock culture of T2 bacteriophage (Presque Isle Cultures, Presque Isle, PA) was prepared by inoculating 100 ml of a mid-log phase culture of *E. coli* ATCC strain 25250 in LB broth with 1 ml of the T2 bacteriophage suspension. The culture was incubated at 37°C rotating at 125 rpm until the bacterial suspension cleared, approximately for 3 h. The lysate was centrifuged at low speed to sediment cell debris, distributed into sterile test tubes and stored at 4°C.

### **Ginger Extract vs. *E. coli* Growth Curves**

An overnight culture of *E. coli* was prepared by inoculating a loop full of bacteria into 100 ml of sterile LB broth in a 250 ml Erlenmeyer flask and placed into a rotary incubator at 37°C rotating at 125 rpm. From four flasks, either 1, 2, 4, or 6 ml of the LB medium was removed and replaced with 1, 2, 4, or 6 ml of the ginger extract prepared in LB broth. A volume of 5 ml of an overnight culture of *E. coli* was inoculated into each flask and placed into the rotary incubator. After indicated times, 4 ml of each culture was removed and the absorbance at 600 nm was measured.

### **T2 Bacteriophage Infection vs. Ginger Concentration**

Volumes of 1, 2, 4 and 6 ml of sterile ginger extract was added to 125 ml Erlenmeyer flasks containing 49, 48, 46 and 44 ml of sterile LB broth. A volume of 5 ml of an overnight culture of *E. coli* in LB broth was inoculated into each flask plus a control flask and incubated on a platform rotating at 125 rpm at 37°C for 2.5 h, approximately one generation time for the bacteria. After 2.5 h of incubation, 1.5 ml of a stock culture of T2 bacteriophage at a multiplicity of infection of 5.0 phage particles per cell was added to each flask and incubated at 37°C at 125 rpm for 10 min. The cultures were centrifuged at 1850×g. for 7 min to sediment the infected *E. coli* cells. Unadsorbed bacteriophage in the supernatant was discarded. The sedimented infected *E. coli*

cells were resuspended in 50 ml of warm LB broth containing the same concentration of ginger extract in which they were cultured and incubated until the bacterial suspension cleared indicating lysis by the bacteriophage. The phage suspensions were placed into sterile centrifuge tubes and stored at 4°C. The lysate was serially diluted in phage dilution buffer (10 mM Tris-HCl, pH 8.3, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.01% gelatin) and a standard plaque assay was performed [15]. Duplicate plates were made for each dilution and an average number of plaques was calculated.

### **Ginger Extract vs. T2 Bacteriophage Adsorption**

To determine if the presence of the ginger extract in the medium affects adsorption of T2 bacteriophage to *E. coli* cells, bacterial cells were inoculated into LB broth and LB broth containing 2 ml of ginger extract and incubated in the shaker incubator at 37°C for 50, 100, and 150 min prior to infection with T2 bacteriophage. The cultures were infected with enough T2 bacteriophage to give a multiplicity of infection of 5.0 plaque forming units per cell. Adsorption was allowed to occur for 10 min, after which the bacteria were centrifuged at 1850×g for 7 min. The supernatant fluid containing unadsorbed bacteriophage was removed, immediately diluted in phage dilution buffer and the T2 bacteriophage concentration was determined as previously described.

### **Time of Addition of Ginger Extract vs. T2 Bacteriophage Yield**

To test the effect of the time of addition of ginger extract on the efficiency of bacteriophage replication, 5 ml of an overnight culture of *E. coli* was inoculated into each of 4 Erlenmeyer flasks containing LB broth and incubated as previously described for 2.5 h. A volume of 2 ml of ginger extract was added to three of the flasks, one flask at 150 min prior to infection, a second flask at 100 min prior to infection, and a third flask at 50 min prior to infection. The cultures were infected with enough T2 bacteriophage to give a multiplicity of infection of 5.0 plaque forming units per cell. Adsorption was allowed to occur for 10 min, after which the bacteria were centrifuged at 1850×g for 7 min. The

supernatant fluid containing unadsorbed virus was removed and the bacterial pellets were resuspended to the original volume with warm sterile LB broth containing ginger extract and incubated at 37°C rotating at 125 rpm. The infection process was continued and the infected cells were incubated on the shaker incubator until the bacterial suspensions cleared. The virus infection process and titration assay were performed on each culture as previously described.

## RESULTS AND DISCUSSION

### Effect of Ginger Extract on the Growth of *E. coli*

The effect of increasing concentrations of ginger extract on the growth of the *E. coli* culture was tested in 250 ml Erlenmeyer flasks each containing 100 ml of sterile Luria-Bertani broth and 1, 2, 4, or 6 ml of sterile ginger extract. The growth curves of *E. coli* in Figure 1 indicate that as the concentration of ginger extract increases, there is a positive correlation with the increase of growth of *E. coli*. At a time of 2.5 h after introduction of the ginger extract, approximately one generation time for the bacteria in this medium, at 1 ml of ginger extract, the *E. coli* cell concentration is 104.6% of the control, at 2 ml and 4 ml of ginger extract, the *E. coli* cell concentration is 117.7% of the control while at 6 ml of ginger extract, the *E. coli* cell concentration is 122.8% of the control.

### Effect of the Concentration of Ginger Extract on T2 Bacteriophage Yield

The effect of increasing concentrations of ginger extract on the yield of T2 bacteriophage in *E. coli* cells was tested. The results in Figure 2 demonstrate that as the concentration of ginger extract increases, the inhibition of T2 bacteriophage replication also increases. Ginger extract at 1, 2, 4 and 6 ml per 100 ml of LB broth decreases the yield of T2 bacteriophage in *E. coli* by 1.66, 36.17, 41.75 and 43.68% respectively.

### Effect of Ginger Extract on Adsorption of T2 Virus

The presence of ginger extract was tested to determine if the efficiency of T2 bacteriophage adsorption to *E. coli* host cells was affected. If ginger extract inhibits T2 bacteriophage from

adsorbing to its host cells, the resulting yield of bacteriophage would decrease. At 50 min post infection, 95.9% of the T2 bacteriophage adsorbed to the *E. coli* cells in the presence of 2 ml of ginger extract per 100 ml of medium, at 100 min, 90.4% adsorbed and at 150 min, 100% of the T2 bacteriophage adsorbed to the *E. coli* cells (Figure 3). The presence of ginger extract in the LB broth medium does not significantly affect bacteriophage adsorption to susceptible bacterial host cells.

### Time of Addition of Ginger Extract

The time of addition of ginger extract was tested to determine if there was an effect on the yield of T2 bacteriophage. If ginger extract inhibited the metabolic activity of treated *E. coli* cells or replicating T2 bacteriophage, the longer the ginger extract was in contact with the treated *E. coli* cells, the greater would be the inhibitory effect. If ginger extract only inhibited the assembly process of bacteriophage replication, there would be no increase in the inhibition, the longer before infection the ginger extract was in the medium. The data in Figure 4 demonstrate that the longer the ginger extract was in contact with the treated *E. coli* cells, the greater was the inhibition of T2 bacteriophage replication. At 50 min of exposure to ginger extract, the T2 yield was 93.75% of the control, at 100 min of exposure, the T2 yield was 87.05% of the control, and at 150 min of exposure, the T2 yield was 79.91% of the control. One or more chemicals present in the ginger extract are inhibiting the metabolic activity of the bacteria and/or the bacteriophage.

### Reversal of Ginger Extract Inhibition of T2 Replication by Glutamine and Nucleosides

Experiments with T2 bacteriophage and adenovirus yield and exposure to the antibiotic 6-diazo-5-oxo-L-norleucine DON, Goldstein *et al.* suggested that the replication of T2 bacteriophage was inhibited by the presence of DON [15, 16]. DON is a structural analogue of glutamine and glutamine is necessary for the *de novo* synthesis of nucleotides. Experiments with excess glutamine and DON indicated that the effect of the addition of extra glutamine reversed the inhibitory effect of DON on the replication of T2 bacteriophage [15] and adenovirus [16]. The effect of excess

glutamine added to *E. coli* cells treated with ginger extract on the inhibition of T2 bacteriophage replication was measured. Concentrations of 7.5, 15, 30, and 45 mM glutamine were added to *E. coli* cells, at the same time 2 ml of ginger extract per 100 ml of LB broth was added. The data in Figure 5 indicate that as the concentration of glutamine increased to 30 and 45 mM, the yield of T2 bacteriophage increased to 113.0 and 128.9% of the ginger treated control *E. coli* cells. This suggests that one or more of the chemicals present in the ginger extract is affecting the same enzyme that DON inhibits and excess glutamine is reversing the inhibition of T2 bacteriophage replication.

DON is an analogue of glutamine and inhibits the aminotransferase enzyme that uses the amino group of glutamine to convert fructose-6-phosphate into glucoasamine [17]. Glucosamine is an integral component for the synthesis of glycoproteins [18]. DON also inhibits the synthesis of nucleotides by preventing the transfer of the amino group

from glutamine to ribonucleotide precursors [19]. Half of all of the nitrogen atoms in the nitrogenous base portions of nucleotides synthesized *de novo* originate from the amino group of glutamine [20].

DON inhibition of T2 bacteriophage was also partially reversed by the addition of nucleosides to DON treated *E. coli* cells. Nucleosides were added to ginger treated *E. coli* cells to see if the inhibition of T2 bacteriophage replication could also be partially reversed. Addition of 1 mM of all four nucleosides caused T2 bacteriophage yield to be 129.9% of the ginger treated control cultures (Figure 6). Addition of 3 and 5 mM nucleosides caused the T2 bacteriophage yield to be 206.1 and 203% of the ginger treated control cultures.

Ginger extract has a similar effect on the replication of T4 bacteriophage in ginger treated *E. coli* cells. At 3 ml of ginger extract in 100 ml of LB broth, T4 bacteriophage is only 54.6% of the control (Figure 7).

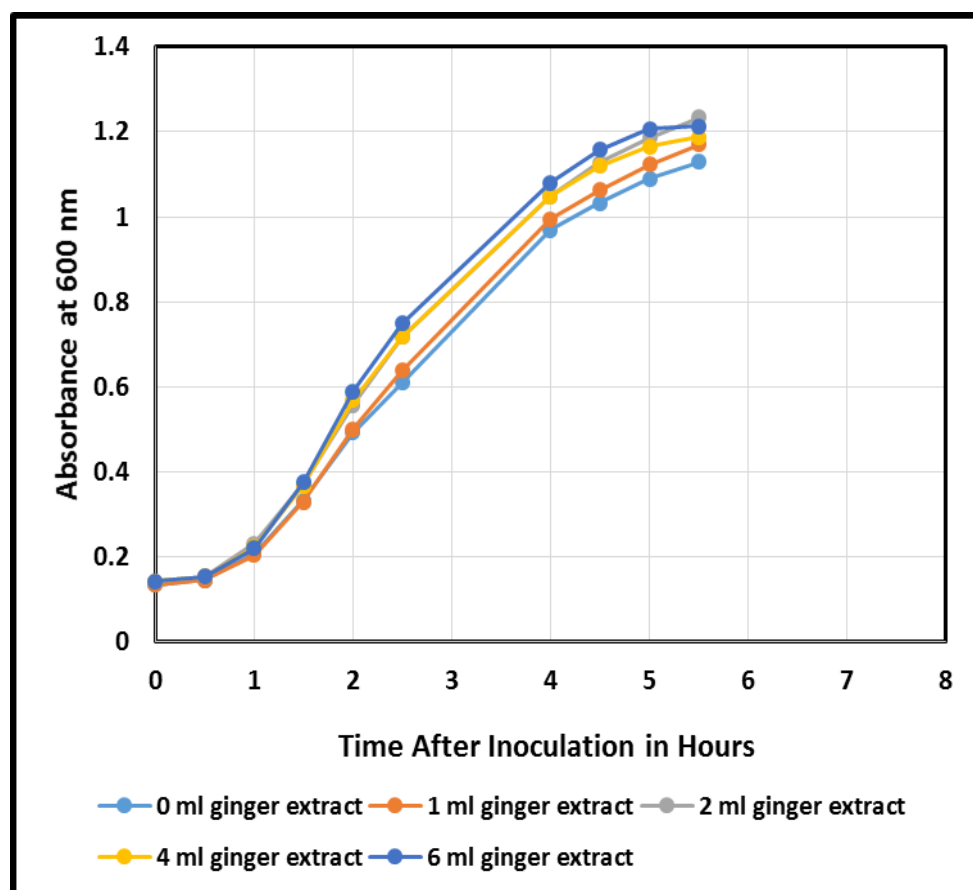
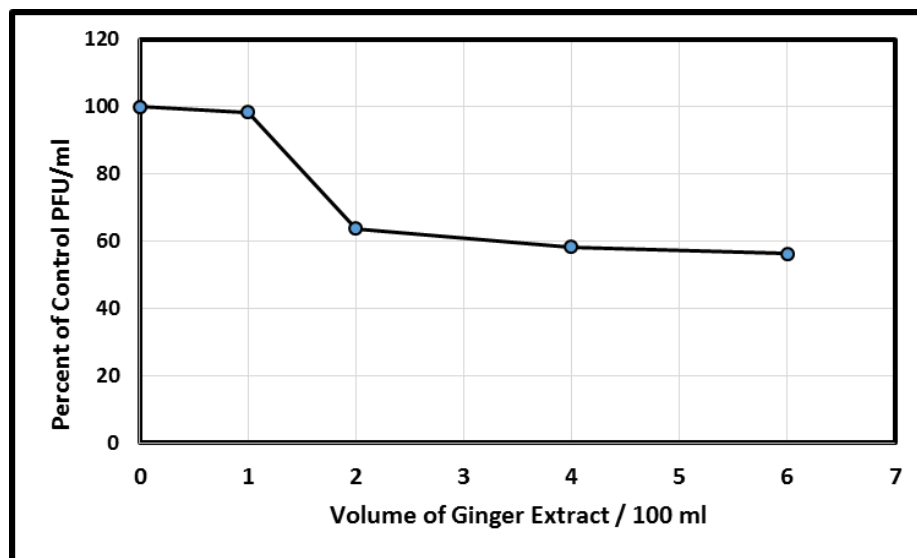
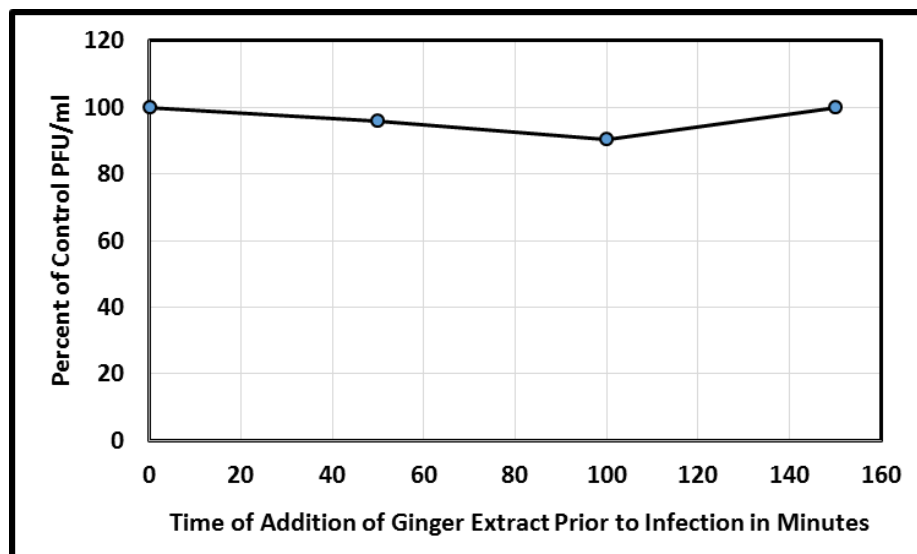


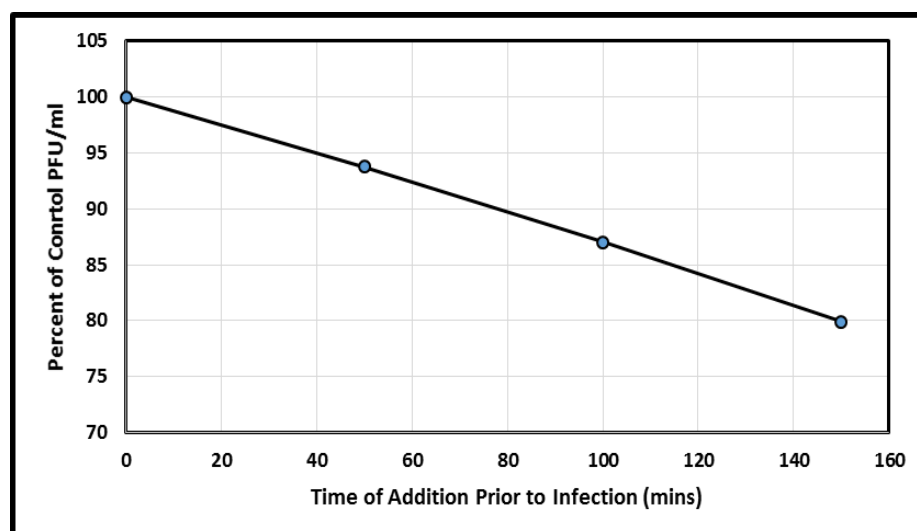
Fig. 1: The Effect of Ginger Extract on the Growth of *E. coli*.



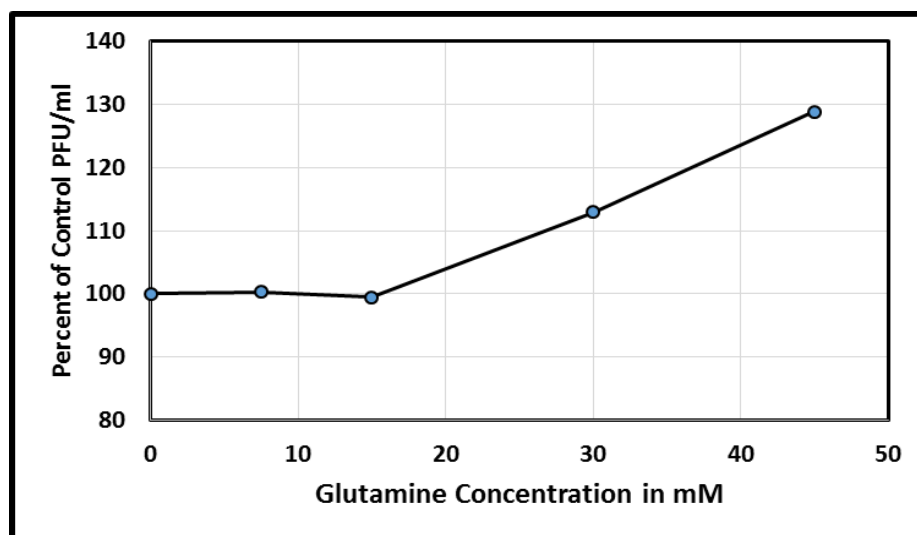
*Fig. 2: The Effect of the Volume of Ginger Extract on T2 Bacteriophage Yield.*



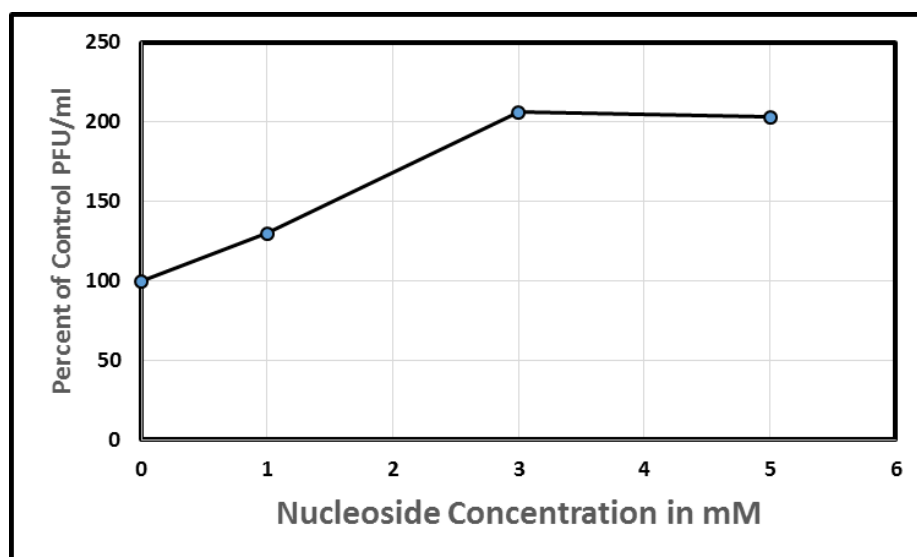
*Fig. 3: The Effect of the Time of Addition of Ginger Extract on Adsorption of T2 Bacteriophage.*



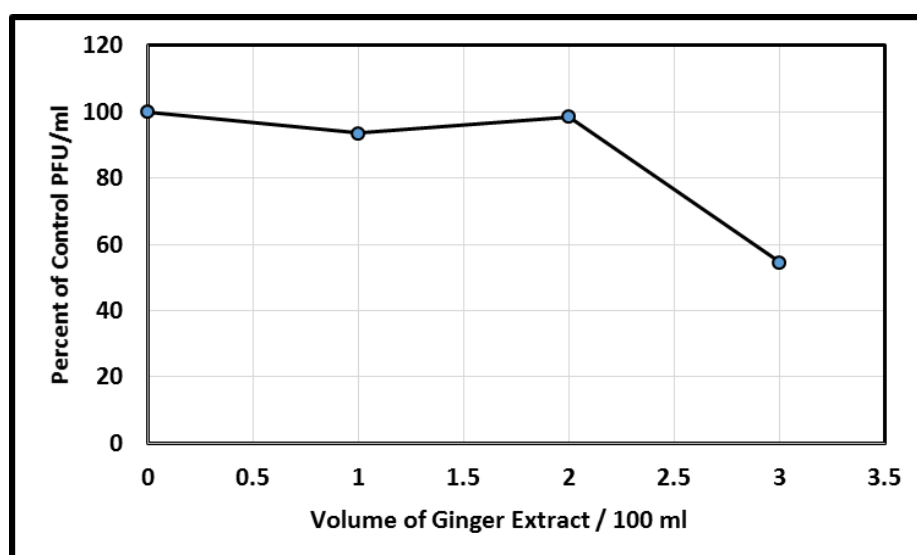
*Fig. 4: The Effect of Time of Addition of Ginger Extract and T2 Bacteriophage Yield.*



**Fig. 5:** The Effect of Excess Glutamine on Ginger Inhibition of T2 Bacteriophage Yield.



**Fig. 6:** The Effect of Excess Nucleosides on Ginger Inhibition of T2 Bacteriophage Yield.



**Fig. 7:** The Effect of Ginger Concentration on T4 Bacteriophage Yield.

## CONCLUSIONS

Exposure of *E. coli* cells to extracts of ginger inhibit the replication of T2 bacteriophage in treated *E. coli*. The addition of excess glutamine and nucleosides partially reverse the ginger extract inhibition of T2 replication. One or more chemicals in ginger extract inhibit the same enzyme as the antibiotic 6-diazo-5-oxo-L-norleucine.

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