

# In Vitro Virucidal Effects of *Allium sativum* (Garlic) Extract and Compounds

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## Abstract

Garlic (*Allium sativum*) has been shown to have antiviral activity, but the compounds responsible have not been identified. Using direct pre-infection inoculation assays, we determined the *in vitro* virucidal effects of fresh garlic extract, its polar fraction, and the following garlic associated compounds: diallyl thiosulfinate (allicin), allyl methyl thiosulfinate, methyl allyl thiosulfinate, ajoene, alliin, deoxyalliin, diallyl disulfide, and diallyl trisulfide. Activity was determined against selected viruses including herpes simplex virus type 1, herpes simplex virus type 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human rhinovirus type 2. The order for virucidal activity generally was: ajoene > allicin > allyl methyl thiosulfinate > methyl allyl thiosulfinate. Ajoene was found in oil-macerates of garlic but not in fresh garlic extracts. No activity was found for the garlic polar fraction, alliin, deoxyalliin, diallyl disulfide, or diallyl trisulfide. Fresh garlic extract, in which thiosulfates appeared to be the active components, was virucidal to each virus tested. The predominant thiosulfate in fresh garlic extract was allicin. Lack of reduction in yields of infectious virus indicated undetectable levels of intracellular antiviral activity for either allicin or fresh garlic extract. Furthermore, con-

centrations that were virucidal were also toxic to HeLa and Vero cells. Virucidal assay results were not influenced by cytotoxicity since the compounds were diluted below toxic levels prior to assaying for infectious virus. These results indicate that virucidal activity and cytotoxicity may have depended upon the viral envelope and cell membrane, respectively. However, activity against non-enveloped virus may have been due to inhibition of viral adsorption or penetration. Additionally, the composition of various commercial garlic products, including garlic powder tablets and capsules, oil-macerated garlic, steam-distilled garlic oils, garlic aged in aqueous alcohol, and fermented garlic oil was determined as well as the virucidal activities of the products against herpes simplex virus type 1 and parainfluenza virus type 3. Virucidal activities of commercial products were dependent upon their preparation process. Those products producing the highest levels of allicin and other thiosulfates had the best virucidal activities.

## Key words

*Allium sativum*, allicin, antiviral, garlic, thiosulfates, virucidal activity.

## Introduction

Garlic, *Allium sativum* L. (Liliaceae), has been used traditionally to treat a number of infectious diseases including those now known to be caused by bacteria, fungi, protozoa, and viruses (1-3). Antibacterial, antifungal, and antiprotozoal effects have been substantiated *in vitro* and found to be due to diallyl thiosulfinate (allicin), methyl allyl thiosulfinate, and allyl methyl thiosulfinate (first named residue linked to the thio, second named group linked to the sulfinate) (3-6). Thiosulfinate compounds are released from garlic cloves after tissue disintegration caused by chewing, cutting, or pressing. Such mechanical action allows alliinase (alliin alkyl sulfenate lyase, EC 4.4.1.4) to convert the (+)-S-alk(en)yl-L-cysteine sulfoxides to the corresponding thiosulfates (shown in Fig. 1) (7). In addition to the endogenous (+)-S-alk(en)yl-L-

cysteine sulfoxide content of garlic cloves, a reserve for the sulfoxides (and hence the thiosulfates) also exists in precursor compounds which are  $\gamma$ -glutamyl-S-alk(en)yl cysteines (8).

The traditional antiviral uses for garlic include treatment of chicken pox, measles, the common cold, and influenza (5). Few confirmatory reports have been published regarding these traditional antiviral uses. Tsai et al. (9) found that a commercial garlic extract from Shanghai, China had *in vitro* antiviral activity against herpes simplex virus type 1 (HSV-1) and influenza B virus, but not coxsackie B1 virus. Studies using mice indicated that aqueous ethanolic extracts of garlic administered orally 15 days before experimental infection with influenza virus AO/PR 8 strain were protective, while administration of this extract at the time of viral infection had no effect