Antiviral Properties of Garlic Cloves Juice Compared with Onion Bulbs Juice Against Potato Virus Y (PVY).

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Abstract: Potato y disease caused by potato virus y (PVY) is widespread wherever potato is grown. PVY affects plants and yields. The effectiveness of extracts from garlic cloves (GE) and onion stems (OE) against potato virus y (PVY) *in vitro* and *in vivo* has been evaluated. GE and OE reduced the *in vitro* and *in vivo* infectivity of PVY to a certain extent, expressed as the number of local lesions induced by PVY on *Chenopodium amaranticolor* plant as a local lesion host. The effect of these extracts and their dilutions (from 10^{-1} to 10^{-3}) was the highest in the crude extract and decreased gradually by increasing the extracts dilutions from 10^{-1} to 10^{-3}. PVY inhibition percentages induced by GE and OE varied according to the time of treatment (1, 2, 3 and 4 days). High percentages of inhibition were recorded for *in vitro* treatment. The highest effect of GE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 63.63 %). While, the highest percentage of inhibition of OE against PVY infectivity was 51.51 % in the crude extract and after 4 days. PVY inhibition of pre-inoculation treatment was higher than that of post-inoculation treatment. In pre-inoculation treatment, the highest effect of GE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 38.89 %). While, the highest percentage of inhibition of OE against PVY infectivity was 33.33 % in the crude extract and after 4 days. In post-inoculation treatment, the highest effect of GE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 33.33 %). While, the highest percentage of inhibition of OE against PVY infectivity was 30.56 % in the crude extract and after 4 days. So, GE was more effective in reducing the local lesions produced by PVY on *Chenopodium amaranticolor* than OE. [Journal of American Science 2010;6(8):302-310]. (ISSN: 1545-1003).

Key words: Potato virus y (PVY), potyviruses, garlic (*Allium sativum*), onion (*Allium cepa*), inhibition.

1. Introduction

Potato virus y (PVY) is one of the most damaging viruses-caused diseases in potato plant (*Solanum tuberosum* L.). PVY is widespread wherever potato is grown. The genome of PVY is 9704 nucleotides long. The flexuous particles of PVY have a model length of 740 nm and width of 11 nm (Robaglia *et al*., 1989). PVY belongs to potyviruses. Potyviruses are important pathogens and cause substantial losses in crop plants of economic importance, such as cereal, millet, fruit, vegetable, sugarcane, oilseed, ornamentals, fodder and pasture, in different parts of the world. Several distinct potyviruses are also known to infect one crop, for instance bean, clover, maize, pea, peanut, potato, soybean, sorghum, tulip, tobacco and cucurbits (Shukla *et al*., 1994). Virus diseases are the major limiting factor of potato production and cause its deterioration (Kurppa, 1983; Omer and El-Hassan, 1992; Jan *et al.,*1994; Arif *et al.,* 1995; Jan and Khan, 1995; Al-Shahwan *et al.,* 1997; Al-Shahwan *et al.,* 1998; Hord and Rivera, 1998; Hamm and Hane, 1999; and Mansour, 1999). Medicinal plants have been widely used to treat a variety of infectious and non-infectious ailments. According to one estimate, 25% of the commonly used medicines contain compounds isolated from plants. Several plants could offer a rich reserve for drug discovery of infectious diseases, particularly in an era when the latest separation techniques are available on one hand, and the human population is challenged by a number of emerging infectious diseases on the other hand. Among several other ailments, viral infections, particularly infections associated with human immunodeficiency virus type 1 (HIV-1) and 2 (HIV-2), and newly emerging infectious viruses have challenged mankind survival. Of importance, a variety of medicinal plants have shown promise to treat a number of viral infections, and some of them possess broad-spectrum antiviral activity. In the past, exploration into the antiviral activity of various promising medicinal plants was limited due to: (a) highly infectious nature of viruses and (b) lack of appropriate separation techniques for the identification of antiviral components from plants. Development of vector-based strategies, in which non-infectious molecular clone of a virus could be used for antiviral screening purposes, and advancement in separation technologies offers promise for medicinal plants usage in modern drug discovery (Muhammad *et al*., 2008). Many investigators have studied the effect of antiviral

activity of garlic or onion on different plant viruses(Dene *et al.,* 1982; Hecht, 1984; Presly *et al.,* 1986; Entwistle *et al.,* 1987 ;Pachauri and hukla, 1988 ; Cheremushkina *et al.,* 1989; Shul'-man, 1989a; Shul'-man, 1989b; Walkey and Antill, 1989; Dijk *et al.,* 1992; Kinghorn and Balandrin, 1993; Pathak, 1993; Dijk and Van, 1994; Patel *et al*., 2000 ; Lot *et al*., 2001; Gurkina, 2002; Thirumalaisamy *et al*., 2003; Cho *et al*., 2010 and Satyakumar *et al*., 2010).

2. Material and Methods:

Extracts of GE and OE were prepared separately in distilled water (1:1w/v). Extracts were diluted by distilled water to 10^{-1}, 10^{-2} and 10^{-3} before use. All experiments were repeated twice. Four replicates were used for each treatment.

1. Virus isolate:

Virus was isolated and identified by Mohamed (1999). Virus inoculum was the crude sap obtained by trituration of frozen leaves of tobacco plants (*Nicotiana tabacum* L. cv.White burley) seedlings showing mosaic and vein banding symptoms. These symptoms developed 14 days after inoculation with a single local lesion obtained from *Chenopodium amaranticolor* leaves that were inoculated with sap extracted from naturally infected potato plants (*Solanum tuberosum* L. cv. Diamond). Inoculation of leaves was carried out by rubbing with finger after their being dusted with carborandum as described by Rawlins and Tompkins, (1936).

2. Preparation of garlic and onion extracts (GE and OE):

Cloves of garlic (*Allium sativum* cv. Balady) and bulbs of onion (*Allium cepa* cv. Giza 20) plants were ground in blender using sterilized distilled water (1:1w/v). The pulp was pressed through two layers of cheesecloth, and then the fluidextract was centrifuged at 1000 rpm for 30 min. The supernatant was collected and stored at -20C (Ismail *et al*., 1989).

3. Effect of garlic and onion extracts (GE and OE) on PVY infectivity *in vitro:*

For testing the effect of garlic and onion extracts for different time intervals(1, 2, 3, and 4 days) on PVY infectivity *in vitro*, 1 ml of the expressed sap containing virus was added to 1 ml of each of garlic and onion extracts, mixed well and allowed to stand for 1, 2, 3 and 4 days. Distilled water was used as a control. Virus-GE and OE mixtures and the control were inoculated into one month old *Chenopodium amaranticolor* at previously mentioned intervals. The developed local lesions were counted and the percentage of inhibition was calculated from the following formula according to Taha and Mousa, (2000):

% Inhibition = (control -treatment) x100/ control

4. Effect of garlic and onion extracts (GE and OE) on PVY infectivity *in vivo:*

4.1. Pre-inoculation treatment:

1 ml of each GE and OE concentrations was rubbed on leaves of *Chenopodium amaranticolor,* then they mechanically inoculated with PVY infected sap (1ml/plant) at different intervals: 1, 2, 3, and 4 days respectively. Distilled water was used as a control.

4.2. Post-inoculation treatment:

The former steps in pre-inoculation were applied except that, virus infected sap was applied first followed by GE and OE treatments

3. Results and Discussion

Garlic has a long history of medicinal use, being mentioned more than 2,000 years ago in ancient Chinese medical literature. Several centuries ago the Egyptians used garlic to treat many disease entities. Aristotle and Hippocrates called attention to the healing powers of garlic, and Pasteur mentioned its medicinal and antibacterial properties. In addition to its well-documented antibacterial properties. Over the past decade, there has been a proliferation of literature on the antimicrobial properties of garlic extract. The principal antimicrobial component of garlic oil is the sulfur compound diallyl thiolsulfinate, which named allicin(Edward and Vincent, 1985).

Onion *(Allium cepa L.)* is a species of the Alinaceae family of great economic importance, and is the second most important vegetable crop in the world. Besides making a significant nutritional contribution to the human diet, onions also have medicinal and functional properties. (Lanzotti, 2006). As with other vegetables, onions contribute to the intake of certain vitamins and minerals, but carbohydrates are the major nutrient fraction, many of which are components of dietetic fibre. Non-structural and soluble carbohydrates form a substantial part of onion dry matter, mainly as fructooligosaccharides (FOS) and monosaccharides (glucose, fructose and sucrose). The non-digestible carbohydrates have recently been shown to be of particular interest for human health. They have a prebiotic effect, improving the intestinal flora, especially the bifidobacteria intestinal conditions against pathogen agents. Onions also demonstrate systemic physiological effects such as decreasing the level of fasting glycemia as well as insulinemia, triglycerides and cholestero. The onion is second only

to the tomato in terms of the agricultural surface area dedicated to its cultivation production. A large increase in onion production has taken place in recent years. The chemical composition of the onion is variable and depends on cultivar, ripening stage, environment and agronomic conditions (Rodríguez *et al.,* 2009).

1. Effect of garlic and onion extracts (GE and OE) on PVY infectivity *in vitro:*

The results obtained from Table (1) and Fig. (1) show an inhibitory effect of GE against PVY infectivity. This effect was the highest in the crude extract and decreased gradually by increasing GE dilutions from 10^{-1} to 10^{-3}. The highest effect of GE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 63.63 %).

Data obtained from Table (2) and Fig. (2) show an inhibitory effect of OE against PVY infectivity. This effect was the highest in the crude extract and decreased gradually by increasing GE dilutions from 10^{-1} to 10^{-3}. The highest effect of GE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 51.51 %).

GE was more effective in reducing the local lesions produced by PVY on *Chenopodium amaranticolor* than OE.

Table (1): Effect of crude and diluted extract from garlic (*Allium sativum* cv. Balady) plants on local lesions number produced by PVY on *Chenopodium amaranticolor in vitro* treatment at different intervals.

Time intervals	Mean number of local lesions								
	Control	Crude extract	% I*	Dilutions			% I*		
				10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
One day	36	23	36.11	25	28	33	30.56	22.22	8.33
Two days	34	19	44.11	21	24	29	38.32	29.41	14.71
Three days	35	16	54.28	18	21	26	48.57	40.00	25.71
Four days	33	12	63.63	14	17	22	57.57	48.48	33.33

% I*: Percentage of inhibition

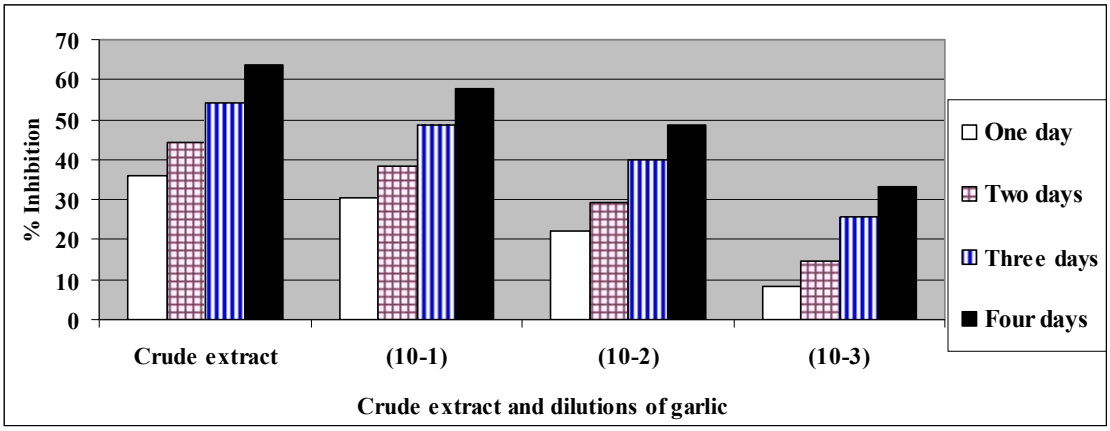

Fig. (1): % of inhibition produced by crude and diluted extract of garlic on PVY infected sap *in vitro.*

Table (2): Effect of crude and diluted extract from onion (*Allium cepa* cv. Giza 20) plants on local lesions number produced by PVY on *Chenopodium amaranticolor in vitro* treatment at different intervals.

Time intervals	Mean number of local lesions								
	Control	Crude extract	% I*	Dilutions			% I*		
				10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
One day	36	26	27.78	28	31	34	22.22	13.89	5.56
Two days	34	22	35.29	24	27	30	29.41	20.58	11.76
Three days	35	20	42.85	22	25	28	37.14	28.57	20.00
Four days	33	16	51.51	18	21	24	45.15	36.36	27.27

% I*: Percentage of inhibition

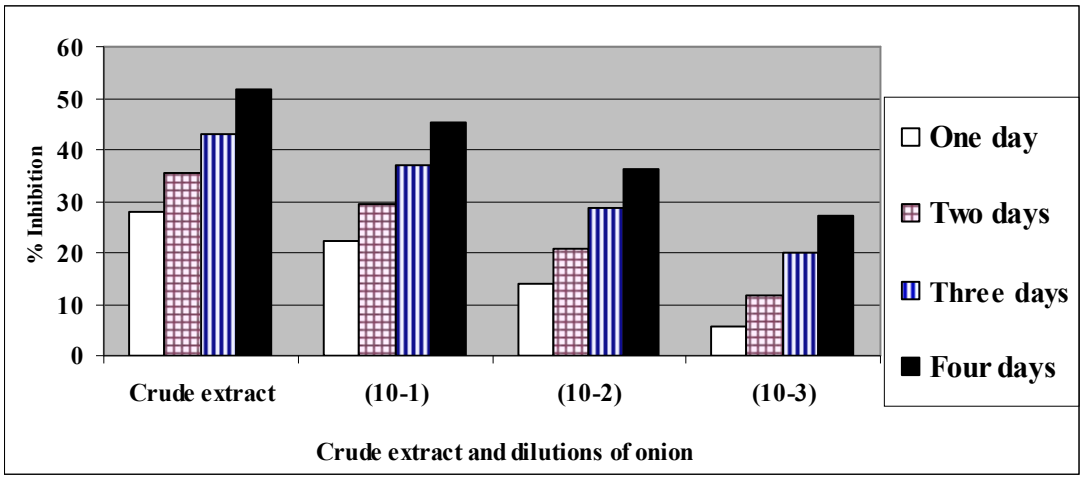

Fig. (2): % of inhibition produced by crude and diluted extract of onion PVY infected sap *in vitro*.

2. Effect of garlic and onion extracts (GE and OE) on PVY infectivity *in vivo:*

Data obtained from Table (3) and Fig. (3) Indicate that better inhibitory effect of GE was obtained by pre-inoculation treatment than post-inoculation one. Effect of GE was the highest in the crude extract and decreased gradually by increasing GE dilutions from 10^{-1} to 10^{-3}. In pre-inoculation treatment, the highest effect of GE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 38.89 %). Also in post-inoculation treatment, the highest effect of GE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 33.33 %).

Similar results were obtained in Table (4) and Fig. (4) using OE. A higher inhibitory effect of OE was obtained by pre-inoculation treatment than post-inoculation one. Effect of OE was the highest in the crude extract and decreased gradually by increasing OE dilutions from 10^{-1} to 10^{-3}. In pre-inoculation treatment, the highest effect of OE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 33.33 %). Also in post-inoculation treatment, the highest effect of OE against PVY infectivity was in the crude extract and after 4 days (percentage of inhibition was 30.56 %). So, GE was more effective in reducing the local lesions produced by PVY on *Chenopodium amaranticolor* than OE.

Table (3): Effect of crude and diluted extract from garlic (*Allium sativum* cv. Balady) plants on local lesions number produced by PVY on *Chenopodium amaranticolor in vivo* treatment at different intervals.

Time intervals	Mean number of local lesions																	
	Pre-inoculation									Post- inoculation								
				Dilutions			% I*					Dilutions			% I*		
	Control	Crude	% I*	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	Crude	% I*	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
One day	33	26	21.21	28	31	32	15.15	6.06	3.03	27	18.18	29	31	32	12.12	6.06	3.03
Two days	35	26	25.71	28	31	34	20.00	11.42	2.85	28	20	30	32	34	14.28	8.57	2.85
Three days	34	24	29.41	26	29	32	23.52	14.70	5.88	26	23.52	28	31	33	17.64	8.82	2.94
Four days	36	22	38.89	24	27	30	33.33	25.00	16.67	24	33.33	26	29	32	27.78	19.4	11.1

% I*: Percentage of inhibition

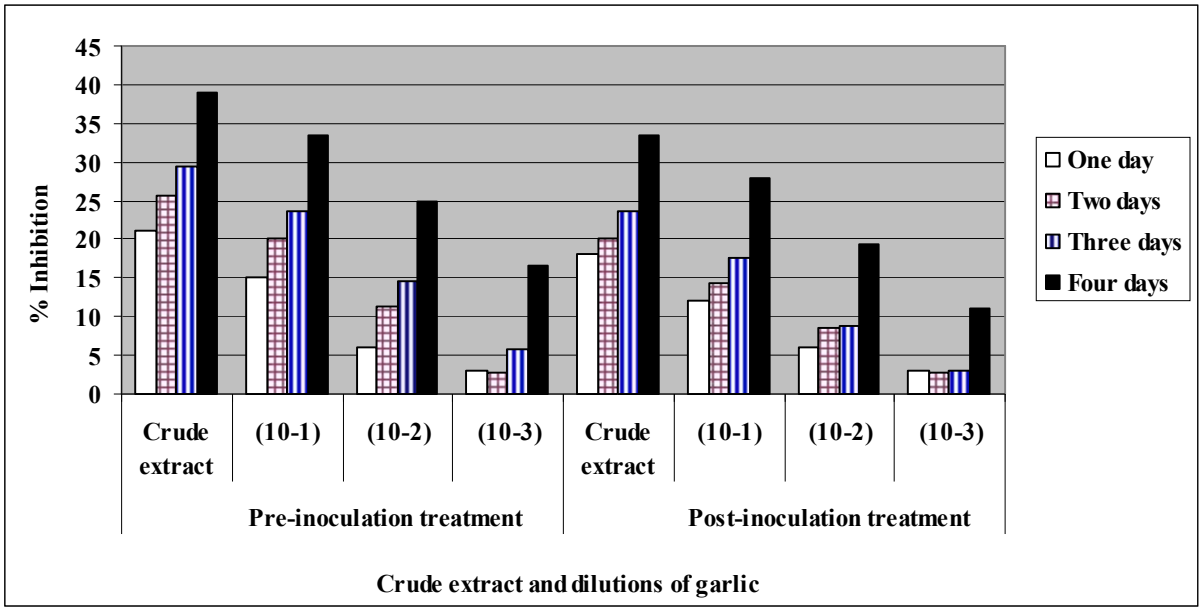

Fig. (3): % of inhibition produced by crude and diluted extract of garlic on PVY infected sap *in vivo*.

Table (4): Effect of crude and diluted extract from onion (*Allium cepa* cv. Giza 20) plants on local lesions number produced by PVY on *Chenopodium amaranticolor in vivo* treatment at different intervals.

Time intervals	Mean number of local lesions																	
	Pre-inoculation									Post- inoculation								
	Control	Crude	% I*	Dilutions			% I*			Crude	% I*	Dilutions			% I*			
				10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}			10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	
One day	33	26	21.2	28	31	32	15.2	6.1	3.0	28	15.2	30	32	32	9.1	3.3	3.3	
Two days	35	27	22.9	29	32	34	17.1	8.6	2.9	29	17.1	31	33	34	11.4	5.7	2.9	
Three days	34	25	26.5	27	30	33	20.6	11.6	2.9	25	26.5	28	30	32	17.6	11.8	5.9	
Four days	36	24	33.3	26	29	32	27. 8	19.4	11.1	25	30.6	27	30	33	25.0	16.7	8.3	

% I*: Percentage of inhibition

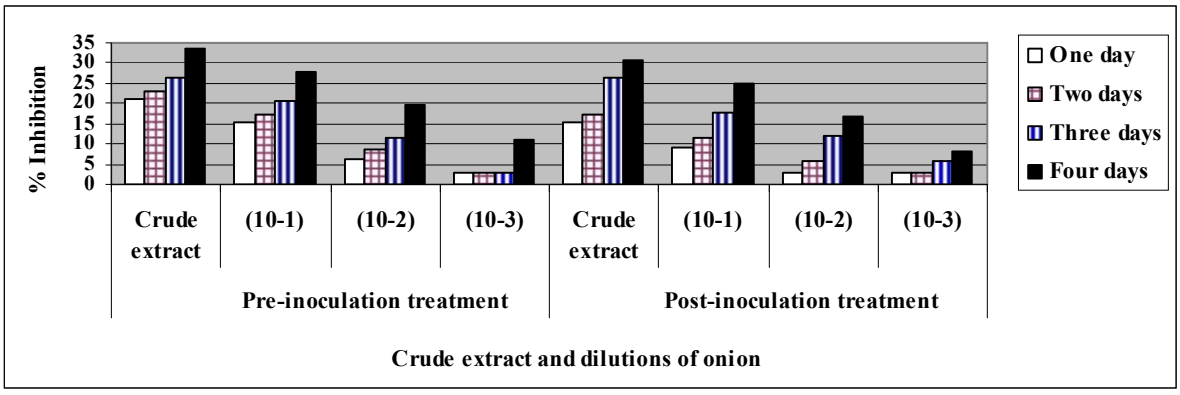

Fig. (4): % of inhibition produced by crude and diluted extract of onion on PVY infected sap *in vivo*.

These findings are compatible with that of Chowdhury and Saha (1985) who tested 15 plant extracts against virus of *Vigna mungo in vitro*, ginger extract gave the highest percentage inhibition after 1 h incubation and turmeric after 2 h. The effects of 4 extracts on the virus *in vivo* were less marked than *in vitro* and onion had the greatest effect, followed by turmeric, ginger and garlic. Othman *et al.,* (1991) investigated the antiviral effect of a crude extract of garlic bulbs on tomato mosaic tobamovirus (TMV) *in vitro*. The extract inhibited the virus when mixed with virus inoculum *in vitro* when used immediately, and after 1 or 2 hours. The extract was thermostable, inhibited local lesions produced by TMV and remained active up to a dilution of 10^{-2}. Viruses which had been treated with the extract were infectious to all varieties of tomato tested but the rate of replication within the host was lower for the extract-treated virus than for the untreated virus. Parida *et al*., (1997) reported that, four plants in traditional medicine in India were assessed for their *in vitro* potential to inhibit polio virus type-3 replication in VERO cells. The ethanolic extract of leaves inhibited polio virus type-3 replication by 99.9%; the aqueous extract was active (99.68% inhibition). Baba *et al*., (1999) showed that, diallyl pentasulfide (DPS), a constituent of aged garlic, dose-dependently inhibited Epstein-Barr virus early antigen (EBV-EA) expression induced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a potent tumour-promoter. DPS did not affect TPA-enhanced 32Pi incorporation into phospholipids of cultured cells, although a low dose of DPS increased 32Pi incorporation. *In vivo*, DPS inhibited TPA-induced epidermal ornithine decarboxylase activity in mouse, and also suppressed the promoting effect of TPA on skin tumour formation in mice initiated with 7,12-dimethylbenz-[a]-anthracene. DPS seems to be one of the anti-carcinogenic constituents of garlic extract. Borek (2001) demonstrated that, Oxidative modification of DNA, proteins and lipids by reactive oxygen species (ROS) plays a role in aging and disease, including cardiovascular, neurodegenerative and inflammatory diseases and cancer. Extracts of fresh garlic that are aged over a prolonged period to produce aged garlic extract (AGE) contain antioxidant phytochemicals that prevent oxidant damage. These include unique water-soluble organosulfur compounds, lipid-soluble organosulfur components and flavonoids, notably allixin and selenium. Long-term extraction of garlic (up to 20 mo) ages the extract, creating antioxidant properties by modifying unstable molecules with antioxidant activity, such as allicin, and increasing stable and highly bioavailable water-soluble organosulfur compounds, such as S-allylcysteine and S-allylmercaptocysteine. AGE exerts antioxidant action by scavenging ROS, enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells. AGE inhibits lipid peroxidation, reducing ischemic/reperfusion damage and inhibiting oxidative modification of LDL, thus protecting endothelial cells from the injury by the oxidized molecules, which contributes to atherosclerosis. AGE inhibits the activation of the oxidant-induced transcription factor, nuclear factor (NF)-kappaB, which has clinical significance in human immunodeficiency virus gene expression and atherogenesis. AGE protects DNA against free radical-mediated damage and mutations, inhibits multistep carcinogenesis and defends against ionizing radiation and UV-induced damage, including protection against some forms of UV-induced immunosuppression. AGE may have a role in protecting against loss of brain function in aging and possess other antiaging effects, as suggested by its ability to increase cognitive functions, memory and longevity in a senescence-accelerated mouse model. AGE has been shown to protect against the cardiotoxic effects of doxorubicin, an antineoplastic agent used in cancer therapy and against liver toxicity caused by carbon tetrachloride (an industrial chemical) and acetaminophen, an analgesic. Substantial experimental evidence shows the ability of AGE to protect against oxidant-induced disease, acute damage from aging, radiation and chemical exposure, and long-term toxic damage. Although additional observations are warranted in humans, compelling evidence supports the beneficial health effects attributed to AGE, i.e., reducing the risk of cardiovascular disease, stroke, cancer and aging, including the oxidant-mediated brain cell damage that is implicated in Alzheimer's disease. Masoomeh *et al*., (2003) used onion and garlic extracts to inhibit fungal growth and keratinolytic activity in *Trichophyton mentagrophytes* fungus as one of the major etiologic agents of human and animal dermatophytosis in Iran and other parts of the world. Eun *et al.* (2005) reported that, a pharmaceutical composition PENNEL comprising garlic oil (GO) and and other compounds, has been used as a curative preparation for patients with acute or chronic viral hepatitis. Chen *et al.,* (2006) illustrated that, the volatile oil was extracted from *Allium sativum*. It was diluted with distilled water to 250 micro g/ml solution and little DMSO was added to make the final concentration at 2 mg/ml. The tobacco mosaic virus (TMV) crude protein solution was added to the volatile oil and observation was done after 2 hours under room temperature. It was found that the volatile oil split the particles of TMV. It strongly inhibited TMV-CP polymerization in vitro, but did not obviously influence

the infectivity of TMV-RNA. The volatile oil also increased the in vitro activities of peroxidase (POD) and polyphenol oxidase [catechol oxidase] (PPO) when sprayed on Nicotiana tabacum . Ji *et al.,* (2007) investigate the anti-listerial effect of garlic shoot juice (GSJ) against the four strains of *Listeria monocytogenes* ATCC 19116, 19118, 19166 and 15313. Various concentrations of (1%, 2.5% and 5%) were used and applied for 0, 4, 7, 10 and 14 days at 4 °C and 0, 6, 12, 18 and 24 h at 37 °C. 5% GSJ showed the strongest anti-listerial effect against all the bacterial strains tested as compared to control at 37 °C. At 4 °C, 2.5% GSJ showed a strong growth inhibitory effect against all the bacterial strains when compared to control after 14 days.

The inhibitory effect of garlic and onion on the virus infectivity may due to many reasons. Serge and David (1991) who concluded that, allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit i) antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli*; ii) antifungal activity, particularly against *Candida albicans*; iii) antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia*; and iv) antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of *E. histolytica*. Yamasaki *et al.,* (1991) examined the effect of allixin, a phytoalexin isolated from garlic, on aflatoxin B_1(AFB_1)-induced mutagenesis using *Salmonella typhimurium* TA100 as the bacterial tester strain and rat liver S9 fraction as the metabolic activation system. The effects of allixin on the binding of [^{3}H]AFB_1 to calf thymus DNA and on the formation of metabolites of [^{3}H]AFB_1 were also determined. Allixin showed a dose-related inhibition of Histidine$^+$ revertants induced by AFB_1. Allixin at 75 µg/ml inhibited [^{3}H]AFB_1 binding to calf thymus DNA and reduced formation of AFB_1-DNA adducts. In addition, allixin exhibited a concentration-dependent inhibition of the formation of organosoluble metabolites and the glutathione conjugates of [^{3}H] AFB1. The data indicate that the effect of allixin on AFB_1-induced mutagenesis and binding of metabolites to DNA may be mediated through an inhibition of microsomal P-450 enzymes. Allixin may thus be useful in the chemoprevention of cancer. Melcher *et al.* (1992) concluded that, many plant species resist systemic CaMV infection by preventing replication or local spread of CaMV, while others solely prevent systemic movement of infection. The inhibitory effect may due to the interference with the protein synthesis of the virus (Gangel, 2002), or may alter the gene transcription (mRNA) of the virus. Virginia (2006) concluded that, onion and garlic among the oldest cultivated plants, are used in the treatment and prevention of a number of diseases, including cancer. These activities are related to the thiosulfinates, volatile sulfur compounds. Besides these low-molecular weight compounds, onion and garlic are characterized by more polar compounds of phenolic and steroidal origin, often glycosilated, showing interesting pharmacological properties. Marta *et al.,* (2007) indicated that, garlic and onion possess many biological activities including antimicrobial, antioxidant, anticarcinogenic, ntimutagenic, antiasthmatic, immunomodulatory and prebiotic activities. Pin *et al.*, (2008) suggested that, the inhibitory effect of onion on the virus infectivity due to its two major flavonoids, quercetin and kaempferol. It is scientifically proven that garlic is effectively used against bacterial, viral, mycotic and parasitic infections. It's also known that garlic is a wonderful plant having the properties of empowering immune system, anti-tumour and antioxidant effects (Goncagul and Ayaz, 2010).

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