



Original Research Article

The inhibitory effects of garlic extract and its fractions against some Enterobacteriaceae sp isolated from sprouted Mung bean

Fahad A. M. Alzowahi*, Ahmed Abu-Taleb, Amani As-Suhbani and T. A. Kadam

Department of Microbiology, School of Life Sciences, S.R.T.M. University,
Nanded, Maharashtra, India

*Corresponding author e-mail: fahadscience@yahoo.com

A B S T R A C T

Keywords

Garlic;
Enterobacteriaceae
sp;
Antibacterial;
fraction
effects.

The present study was to evaluate the effects of raw garlic extract and its fractions against some enterobacteriaceae (*Salmonella* spp, *Enterobacter* spp. and *Escherichia coli*) isolated from sprouted mung bean. The antibacterial activities of raw garlic extract were checked using agar well diffusion method on Muller Hinton agar medium. Raw garlic extract showed inhibitory activities against the bacterial isolates with various sizes of inhibition zones of 15 mm, 23 mm and 24 mm and determined MIC values of garlic extract which were 6.25%, 12.5% and 1.56% against *Salmonella* spp, *Enterobacter* spp. and *Escherichia coli* respectively. Fractions from garlic aqueous extract were obtained by using TLC technique for the separation of bioactive compounds, and column chromatography technique for purification and collection of different fractions separately with solvent system consisting of Chloroform: Methanol: Water (10: 6: 1) respectively. Antibacterial effects of each fraction were performed against the three bacteria individually. The effect of storage temperature was checked and revealed that garlic extract remained stable even after ten days of storage at 4°C, whereas lost its antimicrobial activities after six days of storage at room temperature (30°C).

Introduction

In many countries worldwide, the consumption of seed sprouts has increased in recent decades with the advent of nutraceuticals, phytochemicals (Shetty *et al.*,2003) and the shift of consumer preference toward health foods (Del Rosario, 2003; Pandrangi *et al.*,2003). Mung bean (*Vigna radiata*) sprouts are one of the most common vegetables consumed in some countries due to availability and nutritional value. The

comparable to those obtained from expensive animal and marine sources (Del Rosario, 2003; FNRI, 1997). However, the nutritional quality and the sprouting methods employed by local sprouters make the commodity susceptible to microbial contamination and therefore compromise the safety and quality of the sprouts.

The Commonwealth Scientific and Industrial Research Organization and the

Australian Food Industry Science Center (CSIRO-AFISC, 2000) have reported that the initial microbial flora of the seeds including pathogenic bacteria could increase by 10^1 cfu/g after the soaking period alone and could reach up to 10^6 cfu/g after sprouting, even under hygienic conditions.

Consequently, the consumption of raw or partially cooked sprouts has thus become a major food safety concern (CIDRAP, 2002; OMAF, 2002; Sapers *et al.*, 2002). Taormina *et al.*, (1999) cited that seed sprouts have been implicated in international outbreaks of *Salmonella* spp. and *Escherichia coli* O157: H7 with several reported cases in the United States, Canada and several European countries. Although pathogens like *Salmonella* spp. and *Listeria monocytogenes* have been isolated from mung bean sprouts in Asian countries like Malaysia and Thailand, only outbreaks of *E. coli* O157: H7 have been reported in Japan (Harris *et al.*, 2001; Taormina *et al.*, 1999).

Garlic (*Allium sativum*) has traditional dietary and medicinal applications as an anti infective agent (Ross *et al.*, 2001). Garlic is a common food spice widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases (Rivlin, 2001).

Garlic is thought to have various pharmacologic properties and medical applications. It is mainly consumed as a condiment in various prepared food (Amagase *et al.*, 2001). Garlic is a strong antibacterial agent and acts as an inhibitor on both Gram-positive and Gram-negative bacteria including species of *Escherichia*, *Salmonella*, *Streptococcus mutans*, *Staphylococcus*, *Klebsiella*, *Proteus* and

Helicobacter pylori (Ankri and Mirelman, 1999; Bakri and Douglas, 2005, Reuter *et al.*, 1996). The main antimicrobial constituent of garlic has been identified as the oxygenated sulphur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is usually referred to as allicin.

Allicin is produced catalytically when garlic cloves are crushed and the enzyme allinase (alliin lyase E.C. 4.4.1.4) of the bundle sheath cells mixes with its substrate, alliin, which is released from mesophyll cells (Miron *et al.*, 2000; Curtis *et al.*, 2004). It is one of the principals of freshly crushed garlic homogenates, is a volatile molecule that is poorly miscible in aqueous solutions liquid, responsible for the pungent smell of garlic and is chemically an unstable and highly reactive molecule. It is a short lived molecules, this rather unstable compound has been suggested by Lawson and coworkers to transform rapidly into secondary products (*In vivo*) such as allylmercaptan and others (Koch and Lawson 1996; Lawson and wang, 1993). Therefor this study focused on isolation of some enterobacteriaceae sp from sprouted mung bean and studying the inhibitory effect of garlic extract and its fractions against those bacterial, as well as studying the effect of storage temperature on the activities of garlic extract as antibacterial agent.

Materials and Methods

Collection of samples

Sprouted Mung bean

Sprouted Mung bean was collected in sterilized bags from local market in Nanded- India and brought to the laboratory to be used as a source for the isolation of bacteria.

Garlic

Garlic as a spice was purchased from the vegetable market in Nanded (India), and brought to the laboratory for the extraction of bioactive compounds.

Isolation of bacteria

Sprouted legume seeds were used as a source for the isolation of bacteria, 1 g of sprouted legume seeds was crushed using mortar and pestle. The crushed sample was added in a test tube containing 10 ml Nutrient broth and incubated at room temperature for 24 hours for the enrichment of bacteria. After incubation, a loopful from 24 hours bacterial broth was streaked on plates containing nutrient agar and incubated at room temperature for 24 hours. After incubation, the colonies features of bacteria were reported and each colony was preserved on nutrient agar slant and kept in the refrigerator for further studies.

Identification of bacterial isolates

Various bacteria were grown on the nutrient agar media with different characters. Bacteria were randomly picked up and undergone for further identification processes.

Gram staining technique

Gram staining was done to determine the shapes and the staining of bacteria.

Selective and differential media

Gram -ve short rods bacteria were selected for further identification processes using selective, differential media and biochemical tests. EMB agar, MacConkey agar and Salmonella-Shigella

agar media were used for the identification and differentiation of selected bacteria isolated from sprouted mung bean. Bacteria were individually streaked on the above mentioned media and plates were incubated at room temperature for 24 hours. After incubation distinctive features of each bacterium on the particular media were observed, and the bacteria were selected for biochemical tests.

Biochemical tests

Seven biochemical tests were selected to be performed for the identification of bacteria isolated from sprouted Mung bean (Urea, Methyl Red, Voges Proskauer, Indole, Oxidase, Citrate, triple sugar iron agar).

Preparation of garlic extract

Garlic bulbs were cleaned with tap water and detergent (0.2 % mercuric chloride) for 2 minutes to remove any adhering soil on their surfaces followed by five to six washings with distilled water. 100 g of garlic were taken after removal of their outer skin surfaces and cut into small pieces by sterile scalpel. The small pieces were blended with 100 ml sterile distilled water using sterile warring blender for 5 min at medium speed. The macerates were filtered using sterile funnel and Whatman filter paper. The filtered extract was used for studies within 8 h of extract preparation. Two-fold serial dilutions were prepared from the extract previously prepared, i.e. 50.0, 25.0, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 % (w/v).

Phytochemical screening of garlic extract

The phytochemical screening was performed to determine the chemical

groups of garlic aqueous extract such as (flavonoids, phenols, steroids, tannins, terpenoids, glycosides, reducing sugars and carbohydrates) Egwaikhid *et al.*, 2007.

Flavonoids

One ml of 10% lead acetate solution was added to 1ml of aqueous extracts of spicy samples and observed for the formation of yellow precipitate indicates the presence of flavonoids.

Phenols

Two ml of ferric chloride solution were added to the aqueous extracts of spicy samples and observed for the formation of green or blue colour which indicates the presence of phenols.

Steroids

Two ml of the extracts were dissolved in 2 ml of chloroform and 2 ml of concentrated sulfuric acid was added. The formation of red colour produced in the lower chloroform layer indicates the presence of steroids.

Tannins

About 2 ml of the aqueous extracts of spicy samples was mixed with 2 ml of distilled water and few drops of ferric chloride solution were added. The presence of green precipitate indicates the presence of tannins.

Terpenoids

Two ml of chloroform were added to 0.5 g of the dried aqueous extracts of the spicy samples, and 3 ml of concentrated sulfuric acid were carefully added to form a layer. A reddish brown colouration of the

interface indicates the presence of terpenoids.

Glycosides (Keller- Killani test)

To 2 ml extract, add glacial acetic acid, one drop 5% FeCl₃ and conc. H₂SO₄. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green indicates the presence of glycosides.

Reducing sugars

The aqueous extracts of spicy samples (0.5 g in 5 ml of water) were added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for the colour reaction. A brick red precipitate denoted the presence of reducing sugars.

Carbohydrates (Molisch's test)

The test solution is combined with a small amount of Molisch's reagent (α -naphthol dissolved in ethanol) in a test tube. After mixing, a small amount of concentrated sulfuric acid is slowly added down the sides of the sloping test-tube, without mixing, to form a bottom layer. A positive reaction is indicated by appearance of a purple ring at the interface between the acid and test layers.

Antibacterial effects of extract

The antibacterial effects of garlic extract were checked against the selected bacteria on Muller Hinton agar using agar well diffusion method. 0.1 ml of 24 hours bacterial broth of each bacterium was spread on the agar plates using glass spreader, wells were made using cork borer (6 mm). 100 μ l of garlic extract 25% was added in each well. Plates were incubated at room temperature for 24

hours. After incubation, plates were checked for zones of inhibition (mm).

The effect of temperature on the activities of garlic extract

To evaluate the effect of different temperatures on the antibacterial activities of garlic extract, the extract was divided into two parts, one was kept in room temperature 30°C and the other kept at 4°C for a period of time 1-10 days. Every day the antibacterial activities of each extract were checked against the three bacterial isolates and the results were recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was assayed by recommended method by National Committee for Clinical Laboratory Standards 2010. The Muller Hinton Agar (MHA) media containing various concentration of garlic extract 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, and 0.39 % (w/v), were poured and solidified onto sterile petri dishes to give sterile MHA plates with varying concentration of the extract.

Plates were kept in the refrigerator (4°C) for 24 hours for better diffusion of the extract into the media. After 24 hours, plates were dried at 37 °C for 2 hours. One loopful (diameter 3mm) of overnight grown broth culture of each test bacteria (*Salmonella* spp, *Enterobacter* spp and *Escherichia coli*) were diluted to 10⁻⁶ CFU/ml and spread plated on MHA media. Plates were incubated at room temperature for 24 hours. After incubation the results were reported.

Separation and purification of bioactive compounds

Bioactive compounds from garlic extract were separated by using TLC technique with appropriate solvent system consisting of Chloroform: Methanol: Water (10: 6: 1) respectively. Garlic extract was diluted with sterilized distilled water and loaded on the TLC plates using capillary tube. Plates were run in a chamber containing the solvent system, after running of plates in the solvent system, plates were taken out and allowed to dry at room temperature. Plates were kept in another chamber containing iodine crystal to visualize the bands present on TLC plates. Rf value for each band was calculated.

Purification of bioactive compounds of garlic extract was performed by using column chromatography technique with the same solvent system used with TLC technique starting with less-polar solvent (chloroform) then the polarity was increased by using high polar solvent (methanol and water). Fractions were collected in test tubes 5ml capacity, Rf values were calculated and the fractions which have same Rf values considered as on fraction.

Antibacterial effects of fractions

Antibacterial effects of each fraction were done using agar well diffusion method on Muller Hinton agar. 0.1 ml of each bacteria was spread on the media, wells were made using cork borer, 100 µg of each fraction was dissolved in 1ml sterilized distilled water. 0.1 ml of each fraction was loaded in the wells, and plates were incubated at room temperature for 24 hours. After incubation the results were observed and recorded.

Result and Discussion

Isolation of bacteria

Different colonies of various bacteria were grown on the nutrient agar media each with different characteristics. Random colonies were picked up for further identification.

Identification of bacterial isolates

The results of gram staining revealed that three bacterial isolates B2Mn, B6Mn and B7Mn were gram-ve short rods which might be members of Enterobacteriaceae.

Selective and differential media

Three selective and differential media (macConkey agar, EMB agar and SS agar) were used to differentiate between the three bacterial isolates. B2 Mn isolate gave colourless colonies on MacConkey and EMB agar, while gave colourless colonies with black centre on S-S agar. B6 Mn isolate gave pink colonies on MacConkey agar, purple with dark centre colonies on EMB agar and mucoid pale pink colonies on S-S agar. B7 Mn isolate gave dark colonies with green metallic sheen on EMB agar, pink colonies on MacConkey agar and pink colonies on S-S agar.

Biochemical tests

Based on the biochemical tests, the bacterial isolates B2 Mn, B6 Mn and B7 Mn were identified as *Salmonella* spp, *Enterobacter* spp and *Escherichia coli* respectively (Table 1).

Phytochemical screening

Phytochemical screening results of garlic extracts showed the presence of

flavonoids, steroids, terpenoids, glycosides, reducing sugars and carbohydrates (Table 2).

Antibacterial effects of extract

Garlic extract showed antibacterial effects against the three bacterial isolates which were identified as *Salmonella* spp, *Enterobacter* spp and *Escherichia coli* with inhibition zones of 23mm, 15mm and 24mm respectively (Table 3).

Determination of MIC values

Table (4) shows the MIC obtained, expressed in term of the garlic extract concentration. The MIC values shown were determined by recommended method by National Committee for Clinical Laboratory Standards 2010. MICs of garlic extract were 6.25 %, 12.5 % and 1.56 % against *Salmonella* spp, *Enterobacter* spp and *Escherichia coli* respectively.

The effect of temperature on the activities of garlic extract

Table (5) shows the stability of garlic extract on storage at 4°C and room temperature 30°C changes with the storage time. The antibacterial activities of garlic extract stored at 4°C remained stable and shown the same activities against bacterial isolates even after 10 days of storage, whereas the antibacterial activities of extract stored at room temperature lost its antibacterial activities gradually with the time 1-10 days. This result proves that the components present in garlic extract is sensitive to the temperature and lose their antibacterial activities with time.

Table.1 biochemical tests for the bacterial isolates.

Bacterial isolates	Biochemical tests								
	Urea	Citrate	Indole	MR	VP	TSI medium			
						Slope	Butt	H2S	Gas
B2 Mn	-	+	-	+	-	Red	Yellow	+	d
B6 Mn	-	+	-	-	+	Yellow	Yellow	-	+
B7 Mn	-	-	+	+	-	Yellow	Yellow	-	+

Table.2 Phytochemical screening of garlic extract.

Spice	Screened phytochemicals							
	Flavo	Phen	Ster	Tann	Terp	Gly	R.S	Carb
Garlic	+	+	+	-	+	+	+	+

Flvo (Flavonoids); Phen (Phenols); Ster (Steroids); Tann (Tannins); Gly (Glycosides); R.S (Reducing sugar); Carb (Carbohydrates).

Table.3 Antibacterial effects of garlic extract against selected bacterial isolates.

Garlic Extract	Bacterial isolates		
	Response to the extract		
	Zone of inhibition mm		
	B2 Mn	B6 Mn	B7 Mn
	+	+	+
	23	15	24

Table.4 MIC values of garlic extract against tested bacteria.

Spice	Bacterial isolates		
	MIC values (%)		
	B2 Mn	B6 Mn	B7 Mn
Garlic	6.25	12.5	1.56

B2 Mn (*Salmonella* spp); B6 Mn (*Enterobacter* spp); B7 Mn (*Escherichia coli*).

Table.5 The effect of storage temperature on the antibacterial activities of garlic extract.

Days	Inhibition zones					
	4°C			28°C		
	B2 Mn	B6 Mn	B7 Mn	B2 Mn	B6 Mn	B7 Mn
1	23	15	24	23	15	24
2	23	15	24	23	15	24
3	23	15	24	20	12	20
4	23	15	24	17	12	18
5	23	15	24	10	7	10
6	23	15	24	6	5	8
7	22	13	22	0	0	0
8	22	13	22	0	0	0
9	22	13	22	0	0	0
10	22	13	22	0	0	0

Table.6 Antibacterial effects of different fractions of garlic aqueous extract against the selected bacterial isolates.

Bacterial isolates	Fractions of aqueous garlic extract						
	Bacterial response						
	Zone of inhibition (mm)						
	F1	F2	F3	F4	F5	F6	F7
B2 Mn	12	12	11	12	14	-	12
B6 Mn	11	12	12	-	14	-	12
B7 Mn	14	14	12	-	14	-	13

The separation of bioactive compounds from garlic extract revealed the appearance of seven bands on TLC plates with different Rf values (0.10, 0.27, 0.43, 0.59, 0.70, 0.82 and 0.95) for bands 1, 2, 3, 4, 5, 6 and 7 respectively. Purification of bioactive compounds using column chromatography technique showed the presence of seven fractions from the garlic extract.

Antibacterial effects of garlic fractions

Fractions number 1, 2, 3, 4, 5 and 7 from garlic extract gave antibacterial activities against B2 Mn (*Salmonella* spp.) with

inhibition zones of 12mm, 12mm, 11mm, 12mm, 14mm and 12mm respectively, while fractions number 1, 2, 3, 5 and 7 gave antibacterial activities against B6 Mn (*Enterobacter* spp.) with inhibition zones of 11mm, 12mm, 12mm, 14mm and 12mm respectively, and against B7 Mn (*Escherichia coli*) with inhibition zones of 14mm, 14mm, 12mm, 14mm and 13mm respectively. Fraction number 6 didn't give any antibacterial activity against any bacterial isolates (Table 6).

Sprouted legume seeds are consumed worldwide due to their nutritional value as they are considered the main source of

vitamins, fiber, and mainly protein instead of that obtained from animal and marine sources especially for vegetarian people in some countries like India (Marton *et al.*, 2010; Shah *et al.*, 2011). Different food poisoning outbreaks related to the consumption of raw or partial cooked sprouted legume seeds were reported (Feng, 1997; Taormina *et al.*, 1999; CDCP, 2009; Chris *et al.*, 1999). So, it is necessary to make these commodities safe though it is impossible to make it free of microorganisms but the loading of microorganisms can be reduced and the poisons or toxins produced by such microorganisms are automatically reduced. Spices as flavoring agents in food play important roles in food as antimicrobial agents prevent or inhibit the growth of various microorganisms which might be the causatives of food poisoning agents of out breaks worldwide.

Garlic is used as main spice in different kinds of foods as it gives acceptable test of food and is used for the treatment of different diseases due to its medicinal importance as antioxidant, anticancer, anti-inflammation (Rahman *et al.*, 2012; Othman *et al.*, 2011; Queiroz *et al.* 2009; Londhe *et al.*, 2011; Hosseinzadeh *et al.*, 2011; Seki *et al.*, 2008; Dkhil *et al.*, 2011; Prieto *et al.*, 2011; Ban *et al.*, 2009).

Garlic is one of the most important spicy which has broad spectrum antimicrobial activities against various kinds of microorganisms either pathogens or food spoilage or food poisoning causatives (Alorainy, 2011; Belguith *et al.*, 2010; Shobana *et al.*, 2009; Fatima *et al.*, 2011).

The present study shows the effect of garlic extract and its fractions against tested microorganisms (*Salmonella* spp, *Enterobacter* spp and *Escherichia coli*).

The microorganisms present in the sprouted mung after sprouting process either normal flora of the sprouts or contaminants from different sources, that means the sprouting process enhance the growth of such microorganisms including those causing food poisoning. Based on the results obtained, all bacterial isolates (*Salmonella* spp, *Enterobacter* spp and *Escherichia coli*) were inhibited by garlic extract with different inhibition zones of each bacterium, that means the effect of garlic extract differ from microorganism to another even from the same family. So that it can be possible to eliminate the growth or reduce the amount of such microorganisms by applying garlic extract with appropriate concentrations during the process of sprouting, then the risks due to the consumption of such sprouts will be reduced. Antibacterial activities of garlic extract were shown against the tested bacteria isolated from sprouted mung bean which mean the presence of different compounds rather than allicin having antimicrobial activities against each bacterium with different effect of each fraction. Some fraction affects one bacterium but it is not having any effect against other bacteria. The effect of different temperature on the antimicrobial activity of garlic extract was determined prove that garlic extract remained stable at 4°C even after 10 days of storage, whereas it loses its antibacterial activities after 6 days of storage at room temperature (30°C) (Belguith *et al.*, 2010).

Previous studies reported that (Allicin) undergoes thiol-disulphide exchange reactions and can react with free thiol groups in proteins (Ankri and Mirelman, 1999; Miron *et al.*, 2002). SH containing enzymes so far shown to be inhibited by allicin include: succinic dehydrogenase, urease, papain, xanthine oxidase, choline

oxidase hexokinase, cholinesterase, glyoxylase, triose phosphate dehydrogenase, alcohol dehydrogenase, and cysteine proteases (Ankri *et al.*, 1997; Wills, 1956). Additionally, Focke *et al.*, (1990) provided evidence for specific inhibition of acetyl-CoA synthetase (E.C.6.2.1.1) by allicin which occurred by a non-covalent, reversible binding of allicin to the enzyme.

In contrast to others reports of enzymes inhibition by allicin this effect could not be competed out by thiol reagents such as dithioterythritol (Focke *et al.*, 1990). Allicin's reactivity with enzymes, and its radical-trapping properties and ready membrane permeability, are regarded as the basis of its biological activity (Miron *et al.*, 2000; Rabinkov *et al.*, 1998).

This study concluded that (i) almost bioactive compounds present in raw garlic extract have very nice antibacterial effects against all bacteria isolated from sprouted mung bean, (ii) it is possible to control, eliminate and inactivate the bacteria associated with sprouted mung bean which can be grown during the process of sprouting by using raw garlic extract as antimicrobial agent, (iii) garlic extract is affected by storage temperature as it is remained stable at low storage temperature (4°C) whereas losses its antibacterial activities with the increasing of storage temperature (30°C). This study needs more efforts to determine the actual mechanisms of each compound separately against different types of food poisoning microorganisms.

Acknowledgements

Authors are thankful to Director, School of Life Sciences, and S.R.T.M, University Nanded-431 606 (MS), India, for

providing necessary facilities for successful completing of work.

References

- Amagase, H., B. L. Petesch, H. Matsuura, S. Kasuga and Itakura, Y. 2001. Intake of garlic and its bioactive components. *J. Nutr.* 131: 955S-962S.
- Ankri, S., and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. *Microbes and Infection.* 1: 125-129.
- Ankri, S., T. Miron, A. Rabinkov, M. Wilechek and Mirelman, D. 1997. Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of *Entamoeba histolytica*. *Antimicro. Agents Chemother.* 41: 2286-2288.
- Aneela Fatima., Tauqeer Ahmed., Shaistaj Khan., Farah Deeba and Nasreen Zaidi. 2001. Assessment of antibacterial activity of in vitro and in vivo grown garlic (*Allium sativum* L.). *Pakistan. J. Bot.* 43 (6): 3029-3033.
- Bakri, I. M and Douglas, C.W. 2005. Inhibitory effect of garlic extract on oral bacteria. *Archive.Oral Biol.* 50 (7): 645-51.
- Centers for Disease Control and Prevention. 2009. Outbreak of *Salmonella* Serotype Saintpaul Infections Associated with Eating Alfalfa Sprouts — United States, 2009. *Morbidity and Mortality Weekly Report.* 58: 1-4.
- Commonwealth Scientific and Industrial Research Organization (CSIRO) and Australian Food Industry Science Center (AFISC). 2000. Sprouts and foodborne disease. *Food Safety and Hygiene Bulletin*, Nov 2002. Available from <http://www.dfst.csiro.au/fshbull/fshbull23.htm>. Accessed 14 Nov 2003.
- Center for Infectious Disease Research and Policy (CIDRAP). 2002. FDA cites mung beans sprouts in renewing warning against eating raw sprouts. Available from: <http://www.cidrap.umn.edu/cidrap/content/>

- fs / food-diseases / news / beansprout.html. Accessed 13 Nov 2003.
- Chris, A., Van Beneden., William E. Keene., Robert A. Strang., Denise H. Werker., Arlene S. King., Barbara Mahon., Katrina Hedberg., Alison Bell., Michael T. Kelly., Vijay K. Balan., William R. Mac Kenzie and David Fleming. 1999. Multinational Outbreak of *Salmonella enterica* Serotype Newport Infections. J. American. Med.Asso. 281: 158-162.
- Curtis, H., U. Noll, J. Störmann and Slusarenko, A.J. 2004. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. *Physiol. Mol.Plant Pathol.* 65: 79-89.
- Del Rosario, O.M., 2003. Philippines : a country paper on processing and utilization of legumes. Ch. 12 in *Processing and Utilization of Legumes.* Asian Productivity Organization, Japan.
- Feng, P., 1997. A Summary of Background Information and Foodborne Illness Associated With the Consumption of Sprouts. Center for Food Safety and Applied Nutrition. Washington.
- Food and Nutrition Research Institute (FNRI). 1997. The Philippine Food Composition Tables. FNRI, Department of Science and Technology, Bicutan, Taguig, Metro Manila, Philippines.
- Focke, M., A. Feld and Lichtenthaler, K. 1990. Allicin, a naturally occurring antibiotic from garlic, specifically inhibits acetyl-CoA synthetase. *Federation of European. Biochem.Soci.Lett.* 261 (1):106-108.
- Harris, L. J., L.R. Beuchat, M.E. Parish, T.V. Suslow, E.H. Garrett and Busta, F.F. 2001. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Chap. 3, In *Analysis and evaluation of preventive control measures for the control and reduction/ elimination of microbial hazards on fresh and fresh-cut produce.* Comprehensive reviews in food science and food safety. USA: Institute of Food Technologists (IFT)/Food and Drug Administration (FDA).
- Belguith, H., F. Kthiri., A. Chati., A. Abu Sofah., J. Ben Hamida and A. Landoulsi. 2010. Study of the effect of aqueous garlic extract (*Allium sativum*) on some *Salmonella serovars* isolates. *Emir. J. Food.Agricul.* 22 (3): 189-206.
- Hossein Hosseinzadeh, Javad Behravan, Mohammad Ramezani, Samaneh Sarafranz and Elahe Taghiabadi. 2011. Study of antitumor effect of methanolic and aqueous extracts of *Allium sativum* L (Garlic) cloves using potatodisc bioassay. *Pharmacol.* 1: 881-888
- Jung Ok Ban, Ju Hoon Oh, Tae Myoung Kim, Dae Joong Kim, Heon-Sang Jeong,Sang Bae Han and Jin Tae Hong. 2009. Anti-inflammatory and arthritic effects of thiacremonone, a novel sulfur-compound isolated from garlic via inhibition of NF- κ B. *Arthritis. Res.Therapy.* 11 (5): 1-13.
- Koch, H. P., and Lawson, L.D. 1996. Toxicity, side effects, and the unwanted effects of garlic. In: H. P. Koch and L. D. Lawson (Ed.). pp. 221–228.
- Lawson, L. D., and Wang, Z. L. 1993. Prehepatic fate of the organosulfur compounds derived from (*Allium sativum*). *Planta Medica.* 59 (5): 688-689.
- Londhe V.P., A.T. Gavasane, S.S. Nipate, D.D. Bandawane and Chaudhari P.D. 2011. Role of garlic (*Allium sativum*) in various diseases: an overview. *J. Pharma. Res. Opinion.* 1 (4): 129 – 134.
- Marton, M., Zs. Mandoki., Zs. Csapo-Kiss and Csapo, J. 2010. The role of sprouts in human nutrition. A review. *Acta Univ. Sapientiae, Alimentaria.* 3: 81-117.
- Miron, T., A. Rabinikov, D. Mirelman, M. Wilchek and Weiner.L. 2000. The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. *Biochimica Biophysica Acta (BBA).* 1463: 20–30.
- Miron, T., G. Feigenbalt, L. Weiner, D. Mirelman, M. Wilchek and Rabinikov, A. 2002. A spectro photometric assay for allicin, allin and alliinase (alliin-lyase) with a chromogenic thiol: reaction of 4-

- mercaptopyridine with thiosulfinates. Anal. Biochem. 307:76-83.
- Mohammad, S. Alorainy. 2011. Evaluation of Antimicrobial Activity of Garlic (*Allium sativum*) Against *E. coli* O157:H7. J. Agricult. Veter. Sci. 4 (2): 149-157.
- Ontario Ministry of Agriculture and Food (OMAF). 2002. Sprouted seeds risk assessment. Available from http://assessment=pdf/sproutseed/3seeds_r_a_intro.pdf. accessed 20 Jan 2004.
- Pandurangi, S., M.W. Elwell, R.C. Anantheswaran and LaBorde, L. F. 2003. Efficacy of sulfuric acid scarification and disinfectant treatments in eliminating *Escherichia coli* O157: H7 from alfalfa seeds prior to sprouting. J. Food. Sci. 68 (2): 613-618.
- Rabinkov, A., T. Miron, L. Konsratinovski, M. Wilchek, D. Mirelman and Weiner, L. 1998. The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochimica Biophysica Acta (BBA). 1379: 233-244.
- Rahman, M. M., V. Fazlic and Saad, N. W. 2012. Antioxidant properties of raw garlic (*Allium sativum*) extract. Inter. Food. Res. J. 19 (2): 589-591
- Reuter, H. D., H. P. Koch and Lawson, D.L. 1996. Therapeutic effects and applications of garlic and its preparations. In: H. P. Koch and L. D. Lawson (Eds.). pp. 135–212. Garlic: The science and therapeutic applications of *Allium sativum* L. and related species, 2nd Ed. William and Wilkins, Baltimore, Md.
- Rivlin, R. S., 2001. Historical perspective on the use of garlic. J. Nutrit. 131 (3s): 9515-9545.
- Ross, Z. M., E. A. O'Gara, D. J. Hill, H. V. Sleightholme and Maslin, D.J. 2001. Antimicrobial Properties of Garlic Oil against Human Enteric Bacteria: Evaluation of Methodologies and Comparisons with Garlic Oil Sulfides and Garlic Powder. Appl. Environ. Microbiol. 67: 475-480.
- Sapers, G.M., R.L. Miller, B.A. Annous and Burke A.M. 2002. Improved antimicrobial wash treatments for decontamination of apples. J. Food. Sci. 67: 1886-1891.
- Shetty, K., L. Yuan-Tong, P. McCue, R. G'Labbe, R. Randhir and Chia-Yu, H. 2003. Low microbial load sprouts with enhanced antioxidants for astronaut diet. Society of Automotive Engineers International.
- Siti Fairuz Che Othman, Syed Zahir Iddid, Mustapha Suleiman Koya, 'Aisyah Mohamed Rehan and Kamarul Rahim Kamarudin. 2011. Pertanika. J. Trop. Agricult. Sci. 34 (2): 253 – 261.
- Syed Adil Shah., Aurang Zeb., Tariq Masood., Nadia Noreen., Sayed Jaffar Abbas., Muhammad Samiullah, Md. Abdul Alim and Asim Muhammad. 2011. Effects of sprouting time on biochemical and nutritional qualities of Mungbean varieties. African. J. Agricult. Res. 6(22): 5091-5098.
- Taiichiro Seki, Takashi Hosono, Tomomi Hosono-Fukao, Kahoru Inada, Rie Tanaka, Jun Ogihara and Toyohiko Ariga. 2008. Anticancer effects of diallyl trisulfide derived from garlic. Asia Pacific. J. Clin. Nutrit. 17 (1): 249-252.
- Taormina, P. J., Beuchat, L.R. and Slutsker, L. 1999. Infections associated with eating seed sprouts: an international concern. Emerg. Infect. Dis. 5: 626-634.
- Wills, E. D., 1956. Enzyme inhibition by allicin, the active principle of garlic. Biochem. J. 63:514- 520.
- Yara, S., Queiroz, Emilia Y. Ishimoto, Deborah H.M. Bastos, Geni R. Sampaio, and Elizabeth A.F.S. Torres. 2009. Garlic (*Allium sativum* L.) and ready-to-eat garlic products: In vitro antioxidant activity. Elsevier. Food. Chem. 115:371-374.