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# Antibacterial Activity of *Allium sativum* L. on Pathogenic Bacterial Strains

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**Abstract:** *Allium sativum L*; commonly known as garlic, is a species in the onion family Alliaceae. The aqueous extract and methanol extract of garlic were tested against two Gram positive and three Gram negative bacterial cultures. Of the different extracts tested, the aqueous extract of garlic showed increased inhibitory effect. The maximum antibacterial activity was observed in garlic aqueous extract against *Klebsiella pneumoniae* (8mm) and minimum activity against *Salmonella typhi* (4mm). The growth curve analysis of the test pathogens showed slight reduction on growth compared to control cultures. The percentage reduction of the test cultures were found to be less in 4$^{th}$ hour whereas their activity was effectively reduced on 14$^{th}$ hour. Further separation of active fractions from garlic methanol extract by column chromatography was carried out using Silica gel ($SiO_2$) as adsorbent and subjected to antibacterial assay using agar well diffusion method. The zone of inhibition was compared. Maximum antibacterial activity observed from the crude methanol extract in the column fraction at random number was detected by Thin layer chromatography. These results suggest that *Allium sativum* is a potential plant for controlling pathogenic bacterial strains.

**Key words:** *Allium sativum* · Antibacterial assay · Agar well diffusion method · Column Chromatography

## INTRODUCTION

*Allium sativum* has been used in the history for both culinary and medicinal purposes. They exhibit different properties such as antibacterial, anti-fungal, anti-septic, anti-viral, expectorant, anti-histamine. It has been used as a food additive and also used in traditional remedies in flavonoids and a principle odour allicin (diallyl thiosulphinate). The extracts have been found to anti-histamine. It has been used as a food additive and also used in traditional remedies in flavonoids and a principle odour allicin (diallyl thiosulphinate). The extracts have been found to have a significant protective action against a fat induced increase in serum cholesterol and plasma fibrinogen, fibrinolytic activity and also possess pharmacodynamic properties. Allicin in its pure form, exhibit anti-bacterial activity against a wide range of Gram negative and Gram positive bacteria including multidrug strains of *E. coli*. The increased permeability of allicin through membranes may greatly enhance the intra cellular interaction with thiols was reported [2]. Didry N, *et al*. [3] has tested antimicrobial activity against pathogenic aerobic and anaerobic bacteria from the crude extracts of garlic, onion and shallots. The present study deals with the anti-bacterial activity of different extracts of garlic and isolation of active biological compound from the crude extract by Thin layer chromatography.

## MATERIALS AND METHODS

**Bacterial Pathogens Used for Study:** *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus cereus* and *Streptococcus mutans* were procured from the Department of Biotechnology, Manonmaniam Sundaranar University, SPKCES, Alwarkurichi. The bacterial stock cultures were maintained at 4°C.

**Preparation of Aqueous Extract:** About 10gram of peeled garlic cloves were grinded with sterile distilled water using mortar and pestle. The crude aqueous extract was centrifuged at 8000g for 10 min. The supernatant was collected and preserved aseptically until further use.

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**Preparation of Garlic Methanol Extract:** About 10gram of peeled garlic cloves were grinded using methanol. The finely ground paste was soaked in 100ml of 70% methanol and was wrapped with Para film to prevent the evaporation of volatile compounds. The crude extract was kept in a rotatory shaker for two days at 240-340 rpm. The resultant crude extract was centrifuged at 8000g for 10 min. and the supernatant was collected and stored at -20°C to assess the antibacterial activity against the test cultures.

**Antibiogram Activity:** The antibiogram assay was carried out using well diffusion method against Gram positive and Gram negative cultures. The bacterial inoculums were grown in nutrient broth overnight and about 0.1ml of the test bacterial cultures were spread over on Muller-Hinton agar medium (pH 7.3±0.2 at 25°C) in sterile Petri plates. The plates containing agar medium were bored with syringe puncture with a holding capacity of 8µl and the extract was poured into each well. The extract containing the plates were incubated at 37°C overnight and observed for zone of inhibition.

**Growth Curve:** The growth curve of the test cultures were compared with garlic aqueous extract and non-treated cultures as control. About 2ml of garlic aqueous extract was added to the test cultures inoculated on nutrient broth medium. Optical density was observed for every 2hrs at 540nm for both garlic treated and non-treated cultures using UV spectrophotometer. The growth curves of the treated cultures were plotted against the non-treated cultures and were compared to know the significant inhibitory efficiency of garlic to different test pathogens.

**Determination of Mic (Minimal Inhibitory Concentration):** MIC of garlic aqueous extract against the test cultures were determined by tube dilution method on MH-Broth. The test bacterial cultures were inoculated with various concentrations of garlic aqueous extract, incubated for 24hrs at 37°C which can be observed visually.

**Separation of the Active Fractions from Garlic Extract of *Allium Sativum*:** The crude methanol extract were subjected to active fraction separation by column chromatography using $SiO_2$ as adsorbent. About one tenth bed volume of crude garlic methanol extract was added to the packed column without disturbing the column, whereas 70% methanol was added to the mobile phase solvent. By this all the effective fractions were separated and plated against the test cultures.

**Isolation of Antibacterial Active Compound from Methanol Extraction of *Allium Sativum* by Tlc System:** The active fractions which showed antibacterial activity were subjected to compound separation by TLC with chloroform: acetic acid (1:2) as an eluting solvent. TLC plates were removed from the hot air oven and the samples were spotted on the activated silica gel. After the solvent front reached the top of the plate, it was taken out from chromatographic tank and allowed for air drying. The plates were stained using 50% sulphuric acid by spraying method and observed for separation of active compound.

## RESULTS

The aqueous extract of garlic inhibited the growth of both Gram positive and Gram negative test bacterial cultures. The maximum activity was noted against *Klebsiella pneumoniae* (8mm), *Bacillus cereus* (7mm), *Escherichia coli* (6mm) and *Streptococcus mutans* (6mm) and minimum antibacterial activity against *Salmonella typhi* (4mm).A zone of 2mm was recorded against *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* by methanol. The methanol extract exhibited a zone of 3mm towards *E. coli* and *Klebsiella pneumoniae* and 2mm towards *Salmonella typhi*, *Bacillus cereus* and *Streptococcus mutans,* whereas the autoclaved garlic samples showed no activity against the test cultures.

The growth curve analysis of the test cultures treated with garlic samples indicated slight reduction in growth compared to the control cultures. The percentage reduction of different bacterial cultures treated with garlic extracts were found to be less in $4^{th}$ hour whereas their growth rate effectively reduced after $14^{th}$ hour. The aqueous garlic extract against test bacterial cultures of 20µl in MH broth tubes were of turbid and much difference were not seen on the other added concentration of garlic extract.

The collected different fractions of garlic methanol extract were subjected to antibacterial assay by well diffusion using MHA plates. About 8µl of the fractions were loaded in sterile MHA plates. The inhibitory activity was observed by the zone developed on the plates. The inhibitory effect of different fractions of garlic methanol extract is presented in Table 3. *Streptococcus mutans* was found to exert an inhibitory effect of 7mm in fraction number 83 followed by *Klebsiella pneumoniae* and *Salmonella typhi* with a zone of 5mm at fraction 8 and 50 respectively. An intermediate zone of 4mm was rendered by *E.*coli at fraction 79, *Bacillus cereus* of 3mm at fraction number 74.

Table 1: Antibacterial effect of various garlic extracts on the test cultures as zone formation

| S.No | Cultures | Autoclaved extract (mm) | Water extract ( mm) | Methanol (mm) | Methanol extract (mm) |
|---|---|---|---|---|---|
| 1. | E.coli | 0 | 6 | 2 | 3 |
| 2. | K.pneumoniae | 0 | 8 | 0 | 3 |
| 3. | S.typhi | 0 | 4 | 0 | 2 |
| 4. | B.cereus | 0 | 7 | 2 | 2 |
| 5. | S.mutans | 0 | 6 | 2 | 2 |

Table 2: Percentage reduction of optical density of different bacterial cultures treated with garlic aqueous extract

| | % reduction of optical density at different incubation times (hours) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Organisms | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 |
| E.coli | 91.60 | 84.62 | 91.57 | 94.42 | 92.65 | 73.17 | 55347 | 45.86 | 40.90 | 56.01 | 57.68 | 59.27 |
| K.pneumoniae | 49.11 | 74.60 | 85.96 | 87.00 | 84.58 | 56.73 | 57.72 | 48.21 | 48.91 | 47.70 | 48.91 | 50.93 |
| S.typhi | 74.36 | 39.34 | 80.40 | 89.32 | 87.81 | 87.55 | 74.64 | 59.65 | 58.45 | 57.13 | 60.65 | 59.42 |
| B.cereus | 82.61 | 62.50 | 89.32 | 90.92 | 91.50 | 85.45 | 71.81 | 60.45 | 59.96 | 59.78 | 59.87 | 59.77 |
| S.mutans | 62.07 | 87.94 | 90.55 | 87.87 | 85.76 | 82.26 | 68.75 | 49.97 | 51.36 | 50.69 | 51.37 | 52.50 |

Table 3: Antibacterial assay of column fractions for the test cultures:

| S.No | Bacterial cultures | Fraction numbers | Zone of inhibition (mm) |
|---|---|---|---|
| 1 | E.coli | 79 | 4 |
| 2 | K.pneumoniae | 8 | 5 |
| 3 | S.typhi | 50 | 5 |
| 4 | B.cereus | 74 | 3 |
| 5 | S.mutans | 83 | 7 |

Separation of a single compound from the crude extract of the column fraction at random number was detected by Thin layer chromatographic plates when compared to the crude sample. The overall analysis revealed that the aqueous extract, methanol, methanol extract of garlic were found to be significant in activity against the selective bacterial cultures.

## DISCUSSION

The study of compounds with antibacterial activity have targeted plants with a history of ethno bacterial uses have been reported by Jovel *et al.* Taylor *et al* Sindambiwe. [4,5,6], while Herrera*et al.* [7] reported that only a few studies have targeted on randomly collected plants with localized distribution patterns. Araujo *et al.* [8] reported that antimicrobial properties o f substances on desirable tools in the control of undesirable micro-organisms especially in the treatment of infection and in food spoilage. From the research of De Boer *et al.* [10] it was noted that successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional or practitioners make use of water primarily as a solvent. The result from the present study revealed that methanol extracts of the selected plant was much better and powerful; mainly due to the better solubility of the active compounds in organic solvents. This findings leads to the support of De Boer *et al.* [10] who demonstrated the better solubility of the active compounds in organic solvent.

Lin *et al*, [9] studied the effect of growth media on antibacterial activity. The present study was designed to obtain the preliminary information of the effect of garlic on selective pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Streptococcus mutans* and *Bacillus cereus*). Well diffusion method was used to determine the antibacterial activity than disc diffusion method; which also indicated that the sample volume taken to diffuse can be determined easily in contrast to the disc diffusion method, as it depends on absorbent paper.

In the present study well diffusion method of garlic extracts showed various difference pattern or size, this findings indicated that crude extract possess antibiogram activity significantly for both Gram positive as well as

Gram negative bacterial cultures; which also showed that variation in diameter is mainly due to the cell wall of peptidoglycan and LPS content of bacterial cell wall. Separation and purification of antibacterial compound from garlic performed by column chromatography showed active fractions of various biological compounds for different pathogens; whereas column fractions of 8, 50,74,79,83 showed zone of inhibition against the test cultures. On the basis of zone of inhibition, the present study revealed that the garlic methanol extract possessed significant antibacterial activity. The fractions differentiate the larger, moderate and smaller bio active compounds from garlic extract. Isolation of biologically active compounds from garlic solvent extract revealed that methanol is most suitable for the extraction of antibacterial compound from *Allium sativum.*

Kanaki *et al.* [11] proposed that TLC method was found to be precise, specific, sensitive and accurate and can be used for routine quality control of garlic and its formulations. In the present investigation, presence of a compound was detected from garlic methanol extraction when subjected to TLC for the analysis of column fraction. The result of this work suggested that the compound in active column fraction of methanol extract of garlic has a significant antibacterial effect. These results also demonstrated that *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Streptococcus mutans* and *Bacillus cereus* were effectively inhibitory in aqueous extract of garlic. Further it may be concluded that the different garlic extracts used for the study exhibited inhibitory effect on the test pathogens. A detailed study is needed to assess the physiological role of garlic and its antibacterial activity.

## REFERENCES


1. Han, J., L. Lawson, G. Han and P. Han, 1993. A Spectrophotometric method for quantitative determination of Allicin and total garlic thiosulfonates. Anal Biochem., 225: 157-160.
2. Mirelman, D., T. Miron, A. Rabinkov, M. Wilchek and L. Weiners, 2000. The mode of action of allicin: its ready permeability through phospholipids membranes may contribute to its biological activity. Biochem Biophys. Acta. Jan. 15: 1463(1): 20-30.
3. Didry, N., M. Pinkas and L. Dubreuil, 1987. Antibacterial activity of species of the genus Allium. Pharmazile Oct., 42(10): 687-8.
4. Jovel, E.M., J. Cabanillas and G.H.N. Tower, 1996. An ethno botanical study of the traditional medicine of the Mestizo people of Suni Mirano, Loreta, Peru. J. Ethanopharmacol., 53: 149-156.
5. Taylor, R.S.L., F. Edel, N.P. Manandhar and G.H.N. Towers, 1996. Antimicrobial activities of southern Nepalese medicinal plant. J. Ethanopharmacol., 50: 197-202.
6. Sindabiwe, J.B., M. Calomme, P. Cos, J. Totte, L. Pieters, A. Vlientinck and D. Vanden Berghe, 1999. Screening of seven selected Rwandan plants for antimicrobial an antiviral activities J. Ethanopharmacol., 65: 7-77.
7. Herrera, K.A., C.F. Carson, Riley and D.A. Pereztinherrara, 1996. Antimicrobial activity of extracts from plants endemic to the Canary Islands. Phytother. Res., 10: 364-366.
8. Araujo, C.M.J., M.F. Sousa, Ferreria and C. Leao, 2003. Activity of essential oil from Mediterranean Lamiaceae species against food spoilage yeasts. J. Food Protect. 66: 625-630.
9. Lin. J., A.R. Opuku, M. Geheeb-keller, A.D. Hutchings, S.E. Terblanche, A.K. Jager and M.A. Rassadah, 1998. ASEAN review of biodiversity and Environmental Conservation, (ARBEC) 3: 1-3.
10. De Boer, H.J., A. Kool, W.R. Mizirary, I. Hedberg and J.J. Levenfors, 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania, J. Ethanopharmacol., 96: 461-469
11. Kanaki, N.S. and M. Rajani, 2005. Development and validation of a thin-layer chromatography-densitometry method for the quantization of alliin from garlic (*Allium sativum*) and its formulations. AOAC International., 88: 156.