



Antibacterial effect of *Allium sativum* against organisms isolated from clinical Samples from Cutaneous and Urinary Tract Infections

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Abstract

In this study the antibacterial effect of *Allium sativum* against *Staphylococcus aureus*, *Citrobacterspp*, *Escherichia coli* and *Pseudomonasspp* were determined by agar well diffusion method. The variations in antibacterial effect of fresh garlic, dried garlic and autoclaved garlic were studied. Garlic extracts were prepared with isopropyl alcohol and chloroform. It has been observed that organisms were highly sensitive to dried garlic extract and fresh garlic extract (except *Pseudomonas spp* with zone range of 16mm, 14mm in dry and 14mm, 12mm in fresh chloroform and isopropyl alcohol extract respectively) but autoclaved garlic extract showed no trace of antibacterial effect on any of the organisms. The antibacterial effect of garlic is mainly due to the sulphur compound allicin, yielded from its precursor alliin; present in intact garlic cloves, by allinase enzyme during mechanical grinding or cutting of garlic. Action of allicin is proved to be heat sensitive which justifies the lack of antibacterial effect of autoclaved garlic extract. Currently the bacterial resistance to antibiotics have made it difficult to treat even simple infectious diseases. In this scenario natural substance with considerable antibacterial effect that too with different mode of action is of great medical relevance. The results of this study justify the centuries old traditional medicinal practices with garlic and support and emphasise the use of garlic as therapeutic and health product along with the need and reason to focus more on it.

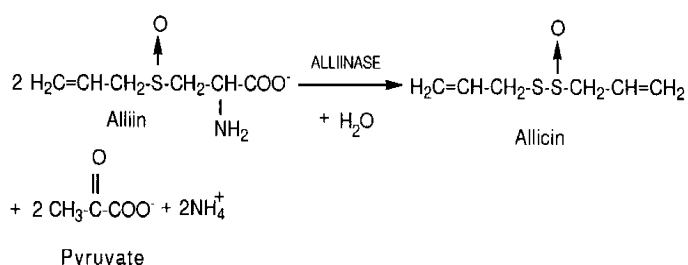
1. Introduction

Garlic (*Allium sativum*) is an erect herb of the *Alliaceae* family, 30 to 60cm tall¹. It is a perennial herb with a characteristic pungent smell. The garlic plants bulb is the most commonly used part of the plant. Bulb is on a

disc like stem, consisting of several segments (cloves), enclosed in a common membrane that is at the base of foliage leaves. The cloves are used for consumption (raw or cooked) or for medical

purpose. *A. sativum* has had an important dietary and medical role for centuries. Some of the earliest references to this medicinal and culinary plant are found on Sumerian clay tablets dating from 2600-2100 BC. Garlic was an important medicine to the ancient Egyptians listed in the medical text Codex Ebers (ca. 1550 BC) especially for the working class involved in heavy labor². Aristotle and Hipocrates called attention to the healing powers of garlic, and Pasteur mentioned its medicinal and antibacterial properties³.

Antimicrobial activity is the ability to either destroy bacteria or inhibit their growth⁴. The principle antimicrobial component of garlic is the sulphur compound diallylthiosulfinate, which Cavallito and colleagues named allicin⁵. An intact garlic bulb does not contain allicin but rather its precursor, allin, which is hydrolysed to allicin, pyruvate and ammonia by the phosphopyridoxal enzyme allinase when tissue of the bulb is disrupted. It is reported that allicin is an inhibitor of sulphhydryl metabolic enzymes and suggests that its antimicrobial properties are due to specific interference with -SH groups⁶.



Now days, microorganisms have developed resistance to several antibiotics and is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and

spreading globally, threatening our ability to treat common infectious diseases and this has produced a vast clinical problem in the management of infectious diseases⁷⁻⁸. This situation enforced scientists to explore new antimicrobial substances from diverse sources such as medicinal plants. Nature has bestowed to humanity the gift of immense therapeutic knowledge with wide variety of medicinal plant. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to humans and environment.

The current study deals with determining the antibacterial effect of *Allium sativum* against *Staphylococcus aureus*, *Citrobacterspp*, *Escherichia coli* and *Pseudomonasspp* by agar well diffusion method. The variations in antibacterial effect of fresh garlic, dried garlic and autoclaved garlic were studied.

2. Materials and Methods

2.1 Materials

Compound microscopes (Observe the morphology of bacteria), Hot air oven, Autoclave (Sterilisation of glassware and media), Incubators (Incubating cultures), Electric balance (Weighing the compounds), Laminar Air Flow (Maintain aseptic conditions), Distillation unit (Sterilisation of water), Mortar and Pestle (Grinding of substances), Glassware's (Test tubes of different sizes, glass slides and coverslips, Petri plates, conical flasks of different capacities i.e. 500 ml. 250 ml,

pipettes of 100- 40 µl).

2.2 Sample collection

The garlic cloves were obtained from Kottayam Town of Kerala state for the research procedure. The cloves were separated and the transparent coverings were peeled manually to obtain the edible portion. A portion of which is sundried another is autoclaved and the rest is left fresh. Mortar and pestle was used to pound the garlic cloves into powder or paste, then sieved and stored in a covered plastic container for further use.

2.3 Isolation of clinical sample

Ten pus and ten urine samples were taken for study and inoculated into blood agar and MacConkey agar and incubated at 37⁰ C overnight. Organisms were isolated. Identification and biochemical characterization done manually by Gram's staining, hanging drop method for motility testing, coagulase test, catalase test, OF test, nitrate reduction test, indole test, MR test, VP test, citrate test, sugar fermentation tests, citrate test, urease test. Cultural characters also noted in various culture media's.

2.4 Preparation of extracts

- Fresh garlic of good quality was purchased from Kottayam market.
- Garlic cloves were separated and peeled off to obtain the edible portion.
- 50g of garlic was weighed out six times for testing in three different conditions, i.e.; dried, autoclaved and fresh.

- For which two portions were chopped and kept under sunlight for few days for dried extract preparation.
- Another two portions were autoclaved and the left two portions were used freshly.
- So as a total we have three categories comprising two portion of garlic each.
- All the six portions were crushed and grinded individually using a mortar and pestle at required time
- One portion from each category was filtered using isopropyl alcohol; individually and so was the other portion with chloroform.
- For that a 250ml sterile conical flask was taken and a funnel was placed on top of the conical flask with folded filter paper on top.
- The grinded garlic with the solvent was added on to the filter paper.
- The setup was left undisturbed for hours.
- The extract was filtered out into the conical flask.
- It was then sealed and kept in the refrigerator for further use and not in the freezer.

2.5 Antimicrobial Susceptibility Testing

These are tests devised to determine the susceptibility of the isolated pathogen to the antimicrobial agent. Media used is Muller Hinton Agar (MHA), having translucent, light amber in colour appearance. Well diffusion

method was adopted here. MHA plates were prepared beforehand using Himedia. Organisms were inoculated as lawn culture on to the plate using a sterile swab and wells were punched. 100µl of the extract was added to the wells using a micropipette and sterile distilled water was used as control. Plates were then carefully placed in an incubator overnight; afterwards clear zones of inhibition were seen and the measurements were recorded.

3. Results

Staphylococcus aureus, *Citrobacterspp*, *Pseudomonas spp*, *Escherichia coli* were the organisms with higher percentage of aetiology and so were taken for study from the 10 pus and urine samples.

Garlic (*A. sativum*) has antimicrobial properties against *Staphylococcus aureus*, *Citrobacterspp*,

Pseudomonas spp, *Escherichia coli*. Dry garlic extract show more antibacterial effect against all the organisms tested followed by fresh extract. Autoclaved extract shows no significant antibacterial effect. Isopropyl alcohol extract of garlic show slightly higher effect than chloroform extract. Allicin is said to be the major component responsible for the antibacterial effect..

Allium sativum has been used traditionally for ages to treat a wide array of diseases. So the results of this study justify the centuries old traditional medicinal practices with garlic and support and emphasize the use of garlic as therapeutic and health product along with the need and reason to focus more on it.

Sensitivity pattern observed for *Citrobacterspp*

Sl no	Sensitivity test using antibiotics		Sensitivity test using extracts					
	Antibiotics used	Zone size	Isopropyl alcohol extract			Chloroform extract		
			Dried	Fresh	Autoclaved	Dried	Fresh	Autoclaved
1	Ciprofloxacin	20	31	26	0	30	22	0
2	Meropenem	20						
3	Amikacin	17						
4	Cefoperazone/Sulbactam	16						
5	Piperacilin/ Tasobactum	16						
6	Cefixime	10						
7	Cefuroxime	8						

Sensitivity pattern observed for *Pseudomonas spp*

Sl no	Sensitivity test using antibiotics		Sensitivity test using extracts					
	Antibiotics used	Zone size	Isopropyl alcohol extract			Chloroform extract		
			Dried	Fresh	Autoclaved	Dried	Fresh	Autoclaved
1	Piperacilin/ Tasobactum	23	16	14	0	14	12	0
2	Ciprofloxacin	21						
3	Amikacin	18						
4	Cefixime	10						
5	Azetreonam	10						
6	Cefuroxime	0						
7	Meropenem	0						

Sensitivity pattern observed for *Escherichia coli*

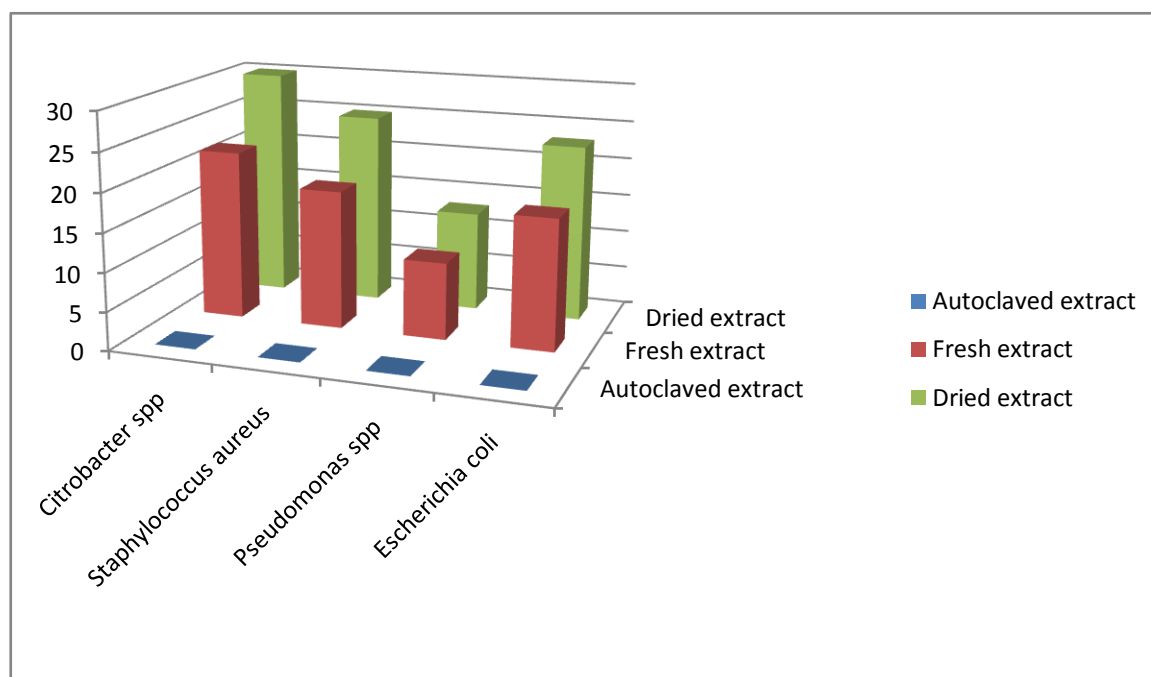
Sl no	Sensitivity test using antibiotics		Sensitivity test using extracts					
	Antibiotics used	Zone size	Isopropyl alcohol extract			Chloroform extract		
			Dried	Fresh	Autoclaved	Dried	Fresh	Autoclaved
1	Chloramphenicol	22	25	22	0	23	19	0
2	Amikacin	18						
3	Cefepime/Sulbactum	14						
4	Ciprofloxacin	10						
5	Levofloxacin	0						
6	Cefuroxime	0						
7	Cefixime	0						

Sensitivity pattern observed for *Staphylococcus aureus*

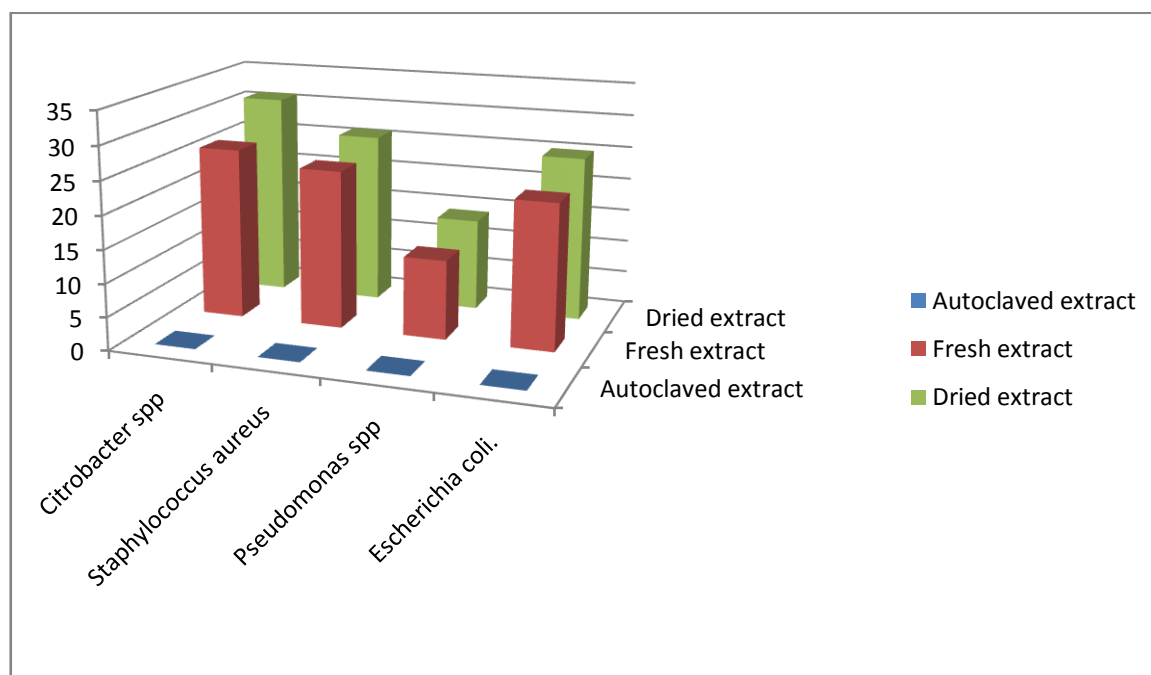
Sl no	Sensitivity test using antibiotics		Sensitivity test using extracts					
	Antibiotics used	Zone size	Isopropyl alcohol extract			Chloroform extract		
			Dried	Fresh	Autoclaved	Dried	Fresh	Autoclaved
1	Cloxacilin	25	27	24	0	25	22	0
2	Cefoperazone/ Sulbactum	24						
3	Clindamycin	20						
4	Ampicillin/Sulbactum	20						
5	Methicillin	16						
6	Levofloxacin	0						
7	Cefurexime	0						
8	Cefixime	0						

Zone of inhibition of the extracts were standardized by comparing with commonly used antibiotics pattern from the Standard chart.

Chloroform extract graph:



Isopropyl alcohol extract graph:



4. Discussion

Garlic has been known for ages to have anti-infective properties against a wide range of microorganisms⁹. This study shows that there is

significant antibacterial effect against *Staphylococcus aureus*, *Pseudomonas spp*, *Citrobacterspp*, and *Escherichia coli*. This indicates that garlic has a broad spectrum of

antimicrobial activity and a wide therapeutic window. The sensitivity of these isolates to garlic extract also implies that the intrinsic bio substances in this extract are naive to the various drug resistance factors of the isolates, which include beta-lactamase expression, increased pyrolydonylarylamidase activity, aminoglycoside-modifying enzymes, and altered ribosomal binding¹⁰. Meanwhile, the antimicrobial potency of garlic has been attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis¹¹. Several studies including those of Rees et al and Kumar and Sharma had previously demonstrated the antibacterial potency of aqueous garlic extracts against enteropathogens such as *Vibrio parahaemolyticus*, *E. coli*, *Klebsiella* spp., *Proteus* spp., and *S. aureus* and anticandidal effects against *Candida* spp¹².

In conclusion, the results of this study have provided scientific justification for the use of garlic extract in health products and herbal remedies against bacterial infections. Therefore, complementary and alternative medicine practices with plant extracts including garlic as a means of decreasing the burden of drug resistance and reducing the cost of management of diseases would be of clinical and public health importance in this country. There is extensive literature on the antibacterial effects of fresh garlic juice, aqueous and alcoholic extracts, lyophilized powders, steam distilled oil and other commercial preparations of garlic. Fenwick and Hanely (1985) undertook a

thorough review of the antibacterial effects of garlic and other allium vegetables up to mid 1984¹³. The antibacterial effects of garlic have been studied by Reuter et al. (1996)¹⁴. The study of Deresse D. A recent study on antibacterial effect of garlic (*Allium sativum*) on *Staphylococcus aureus*: An in vitro study. *Asian J Med Sci.* 2010¹⁵ revealed that antibacterial activity of the garlic extract was heat sensitive, all clinical isolates of *S. aureus* were tested on garlic extract which was autoclaved at 121°C for 15 min. There was no antibacterial effect of garlic in contrast to the work of Shokradeh and Ebadi 2006¹⁶. Tsao S M et al (2001) successfully found sensitivity in *Pseudomonas aeruginosa* garlic oil and four diallyl sulphides¹⁷. This antimicrobial potency disparity of garlic has been attributed to the different concentrations of individually and synergistically active biosubstances in garlic preparations coupled with their interactions with sulfhydryl agents in culture media. This phenomenon has been used to explain the stronger antimicrobial effect of allicin than garlic oil disulfides¹⁸.

5. Acknowledgment

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