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## Evaluation of the Antibacterial Activity of Aqueous Extract of Garlic on *Candida albicans*: A Comparative Study with Conventional Antifungal Drug

	
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### ABSTRACT

The emergence of antibacterial drug resistance is obvious and global confront. Therefore, there is a need for other antibacterials which are new, natural, and plant-based. Garlic (*Allium sativum*) is classified as a member of the family *Amaryllidaceae*. Allicin is one of the active principles of freshly crushed garlic homogenate, having a variety of antibacterial activities. This study was conducted to evaluate the antibacterial effect of garlic against *Candida albicans*. The antibacterial potency of aqueous garlic extract was determined *in-vitro* against *Candida albicans* by agar well diffusion method and broth dilution method. The aqueous extract showed a maximum zone of inhibition of 30mm at 20mg/ml concentration and the lowest zone of inhibition of 16mm at 5mg/ml, compared with clotrimazole which was 28mm at 20mg/ml. The minimum inhibitory concentration (MIC) of aqueous garlic extract was found to be 2 mg/ml. This research concludes that garlic could be used as an effective antibacterial agent for human pathogenic bacteria.

## INTRODUCTION

From the studies, it is identified that 75% of the women will suffer at least one episode of vaginal infection in their lifetime caused by different bacteria such as *Gardnerella vaginalis*, *bacterial vaginosis*, *Candidiasis* and *trichomoniasis* <sup>(1)(2)(3)</sup>. Among these Candidal vaginitis is the second most common vaginal infection <sup>(4)</sup>. Normally, the healthy vagina consists of *Lactobacillus* spp., with *Lactobacillus crispatus*, *Lactobacillus jensenii* and *Lactobacillus iners* being the most prevalent. *Lactobacillus* form a critical line of defence by metabolising the glycogen secreted by the vaginal epithelia into lactic acid. This is highly responsible for maintaining the acidic pH (4.5) of the healthy vagina which is not permissive to the growth of potential pathogens. Marketed formulation (such as tablets, cream, gel, solution, pessaries, etc) mostly belongs to the azole family (clotrimazole, fluconazole, terconazole, miconazole and etc). Frequent use of these antibacterials for prophylactic and therapeutic use has resulted in the development of resistant strains, thus creating a problem in the treatment of infection <sup>(5)(6)</sup>. Moreover, commercial antibacterials are associated with some side effects, whereas herbal antimicrobials there are few side effects, better patient tolerance, and are relatively less expensive. This has directed the interest towards the extraction, characterization of active constituents and subsequent development of dosage forms of herbal origin for the treatment of vaginal infection.

Garlic (*Allium sativum*) is the best known herbal drug with strong antibacterial, antifungal, antimicrobial activity and antiviral<sup>(7)</sup>. It is a perennial, erect, bulbous plant belonging to the family *Amaryllidaceae*. Garlic contains 65% water, and 30% carbohydrates, along with 5% of other bioactive moieties mainly sulphur-containing compounds. <sup>(8)</sup>. Allicin is the active constituent found in garlic responsible for its typical odour and antibacterial activity, which is produced by the enzymatic activity of allinase (a cysteine sulfoxide lyase ) on allin (present in intact garlic) when garlic cloves are cut or crushed <sup>(9)</sup>. Other than Allicin, there are many other important compounds found in garlic extracts such as 1-propenyl allyl thiosulfinate, (E, Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (ajoene), and  $\gamma$ -L-glutamyl-S-allyl-L-cysteine<sup>(10)</sup>. It is found that standardized garlic powder contains 1.3% of all in it. <sup>(11)</sup>

The main target of garlic on Candida cell is not known but it is hypothesized that garlic attacks the candida cell membrane by three mechanisms (1) Oxidises the essential thiols to disulphide (inactivates protein); (2) combines with compounds such as cysteine or

glutathione and inhibits their activity; (3) perform oxidation at the binding to -SH groups at allosteric sites. <sup>(12)(13)(14)</sup>

The objective of the present study was to evaluate the antibacterial activity of aqueous garlic extract (AGE) by broth dilution and well diffusion method using *Candida albicans* and compare its activity with that of clotrimazole.

## MATERIAL AND METHODS

### Material:

The fresh bulbs of *Allium sativum*(Garlic) were purchased from the local market. Clotrimazole was obtained as a gift sample from KGN Pharmaceuticals, Mumbai 400 102, Maharashtra. All other chemicals and solvents used for the research purpose were of analytical grade.

### Methods

#### Identification of Plant

The fresh bulbs of *Allium sativum* L. Belonging to the family *Amaryllidaceae* was identified and confirmed by BLATTER HERBARIUM ST. XAVIER'S COLLEGE, Mumbai-400 001. Fresh garlic bulbs were submitted for authentication.

#### Preparation of garlic extract:

Fresh garlic bulbs were washed thoroughly and cleaned. A fine paste of fresh garlic was used for the preparation of the extract.

#### Cold maceration method:

30gms of fresh garlic cloves purchased from the local market were crushed with 10 ml of distilled water to obtain a fine paste in a grinder or mortar pestle. The mixture was then filtered and further centrifuged at 10000rpm for 20mins. The supernatant was filtered through 0.24µm pore size Whatman filter paper to remove any impurities. The filtrate thus obtained was the aqueous garlic extract (AGE). By subtracting the weight of insoluble material from the weight of the original cloves, the final concentration of the garlic extract in the solution was determined in mg/ml. This liquid extract was used for the antimicrobial assay. Aliquots were stored at 0-2°C in the refrigerator until required <sup>(15)</sup>.

### **Bacteria and growth condition**

Stock culture of *Candida albicans* used in this study was obtained from Patkar-Vardecollege, Goregaon West, and Mumbai. Sabouraud dextrose broth and agar were used to isolate *C.albicans* were obtained from HiMedia Lab. Pvt. Ltd. Subculturing of *C.albicans* was done by inoculating the agar plates and incubating for 48hrs, so as to allow those slow-growing organism to form colonies. *C.albicans* was formed as white colonies.

#### **Liquid culture preparation:**

Liquid bacterial culture was prepared by inoculating a loop full of *Candida albicans* in 5ml of Sabouraud dextrose broth and allowed to multiply by incubating at 37°C for 24hrs to 48hrs. Examine the culture for the presence of growth, which is indicated by turbidity in broth culture.

#### **Agar well diffusion method**

The colonies were transferred from liquid culture to the surface of sabouraud dextrose agar plate with a sterilized straight nichrome wire. The culture was swabbed 3 times by rotating plates at approximately 60° between streaking to ensure even distribution. The inoculated plate was allowed to stand for a few mins but no longer than 15mins before punching the wells in the agar plate. A hollow tube of 5mm diameter was first taken and then heated. It was pressed on an inoculated agar plate and removed immediately after making a well in the plate. So then, four wells were made on each plate. 5mg, 10mg, 15mg and 20mg of AGE were added to the respective wells on each plate. A separate plate for clotrimazole was prepared. All the plates were incubated for 18-24hrs at 37°C. Then the plates were read-only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to the nearest whole millimetre by holding the callipers.

#### **Minimum inhibitory concentration procedure**

The minimum inhibitory concentration (MIC) of the AGE was determined by the broth dilution method.

One set of 1-6 test tubes was labelled as TT1 to TT6. To each test-tube 5ml of Sabouraud dextrose broth was added under sterile conditions. Different concentration of garlic extract ranging from 1 mg/ml to 5 mg/ml was added to marked test tubes except for the first and second test tube. The first and second test tube serves as negative and positive controls. The

tubes were then inoculated with 0.1ml of culture except for the first test tube. All the test tubes were mixed properly and incubated at 37°C for 24hrs. The lowest concentration of garlic extract that completely inhibited the growth of the organism was considered the MIC.

**RESULTS:**

**Antimicrobial activity of AGE by well diffusion method**

The antibacterial activity of aqueous garlic extract and clotrimazole on *Candida albicans* was done by a good diffusion method. Different concentration has a different zone of inhibition as mentioned in the table. No.1 below and shown in Fig.2 and Fig.3.

Table no.1. Antibacterial activity of AGE on C. Albicans by Agar well diffusion method	
Drug concentration (mg/ml)	ZOI (mm)
AGE (5)	16±0.7
AGE (10)	20±0.2
AGE (15)	27±0.4
AGE (20)	30±0.9
Clotrimazole (20)	28±0.6
AGE: aqueous garlic extract; ZOI: zone of inhibition Each value is a mean of ± s.d. (n=3).	

**Inhibitory effect of garlic extract**

Further, the antibacterial activity testing of the AGE was done by the broth dilution method. The AGE exhibited MIC at 2 mg/ml.

Table no.2. MIC of AGE on Candida albicans					
Test tubes	Broth (ml)	Bacteria (ml)	Drug (ml)	Conc. Of drug (mg/ml)	Turbidity
1.	5	-	-	-	-
2.	5	0.1	-	-	+
3.	5	0.1	1ml	1	+
4.	5	0.1	1ml	2	-
5.	5	0.1	1ml	3	-
6.	5	0.1	1ml	4	-

(-) absence of bacterial growth; (+) presence of bacterial growth



Figure 1: Antibacterial activity of AGE against *C. Albicans* using Broth dilution method.

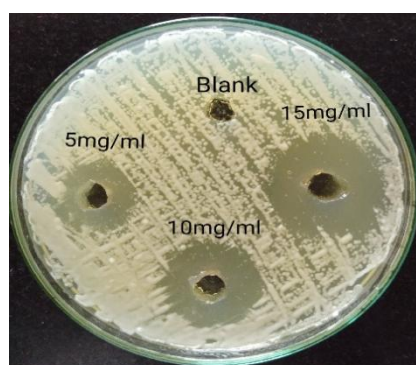


Figure 2: Antibacterial activity of AGE at different concentrations against *C. Albicans* using Agar well diffusion method.

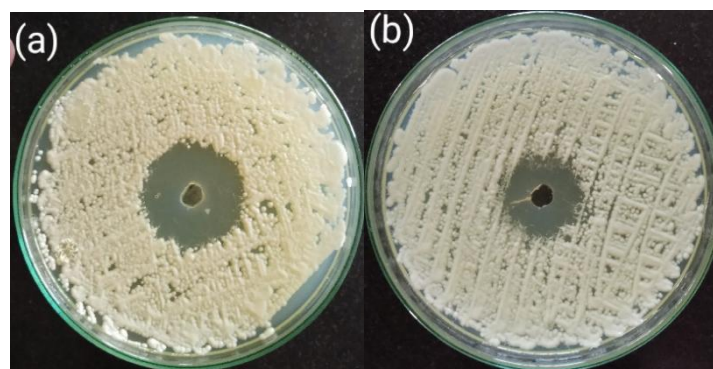


Figure 3: Comparison of Zone of inhibition of Aqueous garlic extract (a) with Clotrimazole(b).

## DISCUSSION:

A healthy vagina is lined with stratified squamous epithelium <sup>(16)</sup> which consists of several hundred species of microbes. Among them, *Lactobacillus* is the leading bacteria that help in maintaining the acidic pH of the healthy vagina by producing lactic acid. A decrease in lactic

acid production leads to an increase in vaginal pH and causes the overgrowth of pathogenic bacteria which are under the suppressed condition in an acidic environment. This imbalance occurs due to many external factors like diabetes, antibacterial therapy, oral contraception, immunosuppressants and pregnancy<sup>(17)(18)</sup>.

Garlic has medicinal, insecticidal, antiproteolytic, antibacterial and antifungal activities<sup>(19)(20)</sup>. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is a principal active compound found in aqueous garlic extract which is produced from allin with the help of the allinase enzyme. The other secondary metabolites such as diallyl sulphide, diallyl trisulfide, allyl methyl sulphide, allyl methyl trisulfide and dipropyl sulfide present in the extract are also found to be responsible for the antibacterial activity. Garlic destroys the bacterial cell membrane and generates oxidative stress in *C. Albicans*. This is because of the activated disulphide bond present in allicin that shows an effect on thiol-containing compounds in bacterial cells<sup>(21)</sup>.

Due to the emergence of resistant strains of *C. Albicans* for azole groups, there is a need to explore an alternative that shows the same action as azole and lesser side effects. In the evaluation of the antibacterial activity of garlic, the aqueous garlic extract has shown a significant potency against the test organism. The area of the zone of inhibition is proportional to the concentration of aqueous garlic extract. Garlic has been known as a potent antifungal agent and compares favourably with antifungal drugs in animal and *in-vitro* studies<sup>(22)</sup>. The area of the non-growth zone increase as the concentration of aqueous garlic extract increases. In this study, garlic showed a maximum zone of inhibition of 30mm at 20mg/ml and the lowest zone of inhibition of 16mm at 5mg/ml. The minimum inhibitory concentration was found to be 2mg/ml when performed by the broth dilution method. The results have shown that the *candida Albicans* were sensitive to aqueous garlic extract. From this, it is clear that garlic can be useful as an antifungal agent against *Candida albicans* which is the leading cause of vaginal infection among women<sup>(23)</sup>.

## CONCLUSION:

With the rise in bacterial resistance to antibacterials, there is considerable interest in the development of other classes of antibacterials for the control of infection. Garlic has been used as a medicine since ancient times and has long been known to have antibacterial, antifungal and antiviral properties. From the current study, it is concluded that there is preliminary evidence for the antifungal activity of garlic extract against *Candida albicans* and

has an equal or better effect against *C. Albicans* when compared with commercial antifungal drug Clotrimazole.

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## AUTHORS CONTRIBUTION

All authors have contributed equally.

## CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

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