

GARLIC AS ALTERNATIVE THERAPY TO TREAT UROPATHOGENE BACTERIA IN WOMEN WITH URINARY TRACT INFECTION IN LOMÉ, TOGO**Banfitebiyi Gambogou^{1,2*}, Abdoul Karim Ouattara², Essodolom Taale^{1,3}, Simplicie D. Karou^{1,2}, Yaovi A. Ameyapoh¹ and Jacques Simpore²**

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ABSTRACT

The urinary tract infection (UTI) is the most common bacterial infection, especially in women. The increased incidence of UTIs, at the last decades have paralleled with the growing emergence of antibiotic resistance. The aim is to evaluate aqueous garlic extract (AGE) susceptibility against multidrug-resistant (MDR) bacteria isolated in urine of women. The investigation of antibacterial propriety and time kill effect of AGE was performed by the well method, microdilution method and spectrophotometer assay. Antibiotics susceptibility assay revealed that the nine MDR bacteria had high resistance against Amoxicillin/clavulanic acid (100%) and Erythromycin (100%), Cefotaxime (83.33%) and Ceftazidime (83.33%). AGE exhibited potent antibacterial activity against the nine MDR bacteria tested. In Gram-negative bacteria, the inhibition diameters ranged from 20 ± 3 to 32 ± 4 mm, with Minimum Inhibitory Concentrations (MICs) ranging from 10% to 12.5% (w/v) and Minimum Bactericidal Concentration (MBCs) was 12.5 % (w/v). Gram-positive bacteria exhibited diameters ranging from 38 ± 2 to 45 ± 1 mm; MIC and MBC values ranged from 05 to 10 % (w/v) and were found more susceptible than Gram-negative bacteria. To conclude, this investigation shown that AGE have high potential antibacterial to use as an alternative to treat women UTIs.

KEYWORDS: Garlic, alternative therapy, Antibiotics, Uropathogenic bacteria, Women, Lomé.

INTRODUCTION

The urinary tract infection (UTI) is the most common bacterial infection, especially in women, since it is estimated that 50% of women will have at least one urinary infection during their lifetime.^[1] Economic burden of the society, the UTI is responsible for nearly seven million external visits and one million emergency visits, resulting in over 100 000 hospitalizations annually in the United States.^[2] In community settings, it mainly affects sexually-active women, while in the middle hospitable, it is the elderly and the carriers, which are the most affected. In general, UTIs are associated with a single bacterium. Bacteria most often involved in UTIs acquired in a community are *Escherichia coli* and *Staphylococcus saprophyticus*, responsible for more than 80 % and 10 % to 15 % of UTI, respectively.^[3,4] Occasionally other microorganisms, such as *Klebsiella spp.*, *Proteus mirabilis*, and *Enterococcus faecalis*, can be involved.^[1,5] Despite the increased incidence of UTIs globally, the last decades have paralleled the growing

emergence of antibiotic resistance. Antimicrobial resistance in UTIs has reported worldwide and increasing rates of resistance is a growing concern in both developed and developing countries.^[6,7] Multidrug resistant, pose treatment problem resulting in high morbidity, high mortality, and increased health care costs.^[8] The multidrug resistant (MDR) strains are major public threat worldwide^[9] and are highly prevalent in Togo.^[10,11] Antimicrobial resistance is a leading concern and efforts should be make to ensure an appropriate duration of therapy for symptomatic infections.^[12] Although, in several countries, medical herbal are already used in their health care.

In all developing countries, such as Togo, medicinal plants are the most widely used means, especially in rural areas, for solving public health problems. At present, the situation regarding the abundance and availability of plants in general and medicinal plants in particular remains poorly known. The search on

medicinal plants, generally devoted to ethnobotanical surveys, made it possible to draw up a non-exhaustive list of plant species used in traditional medicine by the populations.^[15,14] The study of biological and chemical properties has shown that Togolese flora has a real therapeutic and nutritional potential that can be used to treat or prevent many diseases. This is the case of Karou *et al.*^[15] and Tchacondo *et al.*,^[16] who have shown the potential of some medicinal plants in the treatment and control of infections. These infections are one of the leading causes of death and economic loss. Plants have an interest in human health for their antibacterial,^[15,17] antifungal,^[18] and antiparasitic activities.^[19]

Allium sativum commonly known as garlic have documented and valued for their spicy and medicinal qualities by many cultures around the globe for many years.^[20] Garlic is the edible bulb of the lily (*liliaceae*) family, which is widely used as a spice in flavoring, as foodstuff, and condiment around the world.^[21] Garlic has been used to treat bacterial related diseases such as pile, cough and rheumatism; also, to alleviate tumor, cardiovascular diseases and ageing.^[22] Now, garlic has been known for his antibacterial, antifungal, and antiviral properties.^[23,24] Garlic have been shown to have a wide spectrum antibacterial activity, including effects on *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Mycobacterium*, and *Helicobacter* species.^[24,25]

No data on the antimicrobial activity of garlic grown in Togo and its use in the treatment of urinary tract infections exists in the literature. Then objective of this study was to evaluate the antibacterial activity of fresh garlic bulbs against uropathogenic bacteria isolated from the “Centre Hospitalier Regional - Lomé Commune” in women with urinary tract infection in the aim to propose the use of garlic in the treatment of urinary tract infections caused by multidrug resistant bacteria.

MATERIALS AND METHODS

Plant Material

Fresh bulbs of *Allium sativum* L., commonly known as garlic (“AYO” in the local name) were purchased from “ADWLATO” market, the famous spices market of Lomé, capital city of Togo and stored at Laboratory temperature 24 ± 1 °C.

Ethics

Our studies performed did not have direct links with women. We used only bacteria strains isolated in urine of women bring for analysis. The investigator of the study have not information about the origin of urine.

Bacteria strains

Nine bacterial strains were isolated from the urine of women hospitalize or come for consultation at “Centre Hospitalier Regional – Lomé” (Togo). These bacteria strains are *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella oxytoca*, *Klebsiella*

pneumoniae, *Citrobacter freundii*, *Proteus mirabilis*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Enterococcus faecalis*. Bacteria strains tested were identified by conventional methods of microbiology and used API 20E and API STAPH (Biomérieux, Paris, France).

Antibiotics susceptibility of uropathogenic bacteria strains

An inoculum of each strain was prepared, and the turbidity of the suspension was adjusted to achieve 0.5 McFarland with the absorbance range of 0.09 by UV-Vis spectrophotometer at wavelength of 620 nm. The antibiotic susceptibility were tested by Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute^[26] using Mueller Hinton agar medium (Oxoid, Hampshire, England). The antimicrobial agents tested were Gentamicin (10 µg), Imipenem (30 µg), Ceftazidime (30 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Amikacin (30 µg), Amoxicillin + Clavulanic acid (20/10 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg) Vancomycin (30 µg), Kanamycin (3 µg), Pefloxacin (15 µg), Lymcomycin (15 µg), Erythromycin (15 µg), Chloramphenicol (30 µg) (Oxoid, England). Resistance data were interpreted according to Clinical and Laboratory Standards Institute.^[26]

Antibacterial Activity of garlic

AGE susceptibility against the nine multidrug resistance bacteria by well diffusion

After adjusting the inoculum to a 0.5 McFarland unit turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of Muller Hinton agar was swabbed three times, rotating plates approximately 60 °C between streaking to ensure even distribution. The inoculated plate was allowed to stand for at least 3 min but no longer than 15 min before punching the wells in the agar plate. A hollow tube of 5 mm diameter was taken and heated. It was pressed on the inoculated agar plate and removed immediately after making a well in the plate. Likewise, four wells were made on each plate. 50 µl of AGE were added into the respective wells on each plate, and one of the well filled with sterile water and served as negative control. The plates were incubated within 15 min of compound application for 18-24 h at 37 °C. The antibacterial activity of AGE was determined based on clear inhibition zone around the wells. The results are shown as mean values from triplicate measurements.^[26]

Determination of Minimum Inhibition Concentration by Microdilution Assay

The Minimum inhibitory concentrations (MICs) were determined using standard Microdilution test of according to the recommendation CLSI.^[26] The MDR bacteria isolates were grown overnight to log-phase in Luria-Bertani (LB) broth with shaking (170 rpm) at 37 °C and suspended in sterile phosphate buffer saline (PBS) to a concentration of cells equal to 0.5 McFarland

with the absorbance range of 0.09. After that, they were diluted in Mueller Hinton broth and to obtain a final inoculum concentration of 2×10^5 CFU/mL. Subsequently, 100 μ L of bacterial suspension and 100 μ L of two fold serial AGE dilution (at concentrations ranged from 5 to 640 mg/mL) in MHB or 100 μ L MHB without AGE were added into 96-well polystyrene microtiter plates and the absorbance of each well was read at 600 nm. The plate was incubated at 37 °C for 24 h and the absorbance was read again at the same wavelength. All experiments were performed in triplicate. The MIC of AGE was defined as the lowest concentration that completely inhibited the bacterial growth.

Determination of Minimum Bactericidal Concentration

For determining The minimum bactericidal concentration (MBC) values, an aliquot (10 μ L) of MIC mixtures that showed no growth was inoculated onto Mueller Hinton plates and incubated at 37 °C for 24 h^[26]. MBC values were read as the lowest concentration of AGE required for a 99.9% reduction in the viable strains.

Time-Kill Assay

The time-kill effect of AGE were performed in microplate. The each column of microplate were prepared with 100 μ L of inoculum bacteria. The line one of microplate was set as a control. After which 100 μ L of the AGE 1 mg. ml⁻¹ (AGE1, AGE2 and AGE3) were added in the line two to four. Microplate tube were incubated at 37°C and optical density (OD) at 620 nm was observed at interval of every three hours up to 24 hours. The results obtained are the average of the three (AGE1, AGE2 and AGE3).

Statistical Analysis

The results were reported as mean \pm standard deviation (\pm SD). comparisons of means were performed after each analysis of variance (ANOVA) to distinguish the antibacterial effect of AGE against negative and positive Gram bacteria.

RESULTS

Antibiotic Sensitivity

The table 1 and figure 1 shows the antibiotics profile of several UTI bacterial strains isolated from urine of women in Lomé. It show that the nine bacteria tested exhibit-varying degrees of resistance of standard antibiotics used in this study. The most sensitive antibiotics against the nine bacteria are Amikacin (83.33%), Gentamycin (77.78%), Imipenem (77.78%) and Nalidixic acid (55.56%). However, some antibiotics are poor sensitive against the bacteria tested. The most resistant antibiotic are Amoxicillin/clavulanic acid (100%) and Erythromycin (100%), Cefotaxime (83.33%) and Ceftazidime (83.33%). It's reveal that uropathogene bacteria strains in this study present high levels of multidrug resistances.

Antibacterial effect of garlic extract against all bacteria isolates

The results of inhibitory diameters, MICs and MBCs of garlic are show in figure 2 and Table 2. Garlic have reveal an antibacterial activity against all tested bacteria (Figure 2); the diameters of inhibition ranged from 20 ± 3 mm (*Klebsiella oxytoca*) to 45 ± 1 mm (*E. faecalis*) against the nine multidrug resistance bacteria tested. Garlic extracts displayed varying degrees of activity against uropathogene bacteria. It can be see that garlic extract MIC and MBC ranging from five % (w/v) for Gram-Positive bacteria to 12.5 % (w/v) for Gram-Negative bacteria. This MICs and MBCs inhibit the growth of all tested bacteria.

Time kill Curve

Figure 3, represent the time kill effect against the nine MDR bacteria strains tested. It were performed by spectroscopic method. According to the results (Figure 3), the untreated (control) bacterial suspension shows time dependent increased absorbance at 600 nm indicates growth of bacteria; comparatively the treat (AGE) bacterial suspension shows less absorbance. Thus shows different bactericidal action of AGE. *Staphylococcus saprophyticus*, *Staphylococcus aureus* and *Enterococcus faecalis* are destroy after nine hours of incubation, but *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Proteus mirabilis* and *Enterobacter aerogenes* are destroy after 24 hours. That is reveal, AGE are more killing potential of GPB than GNB.

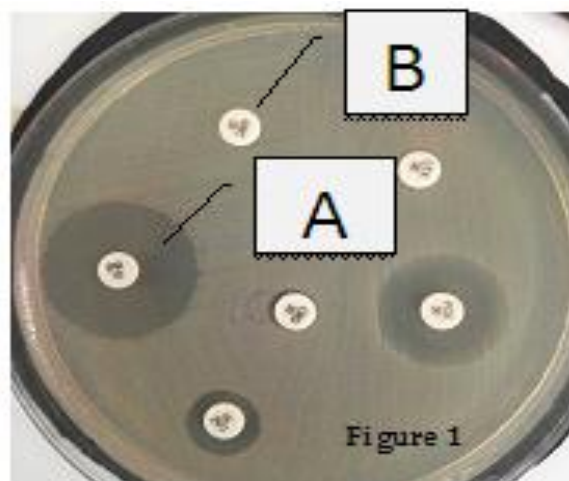
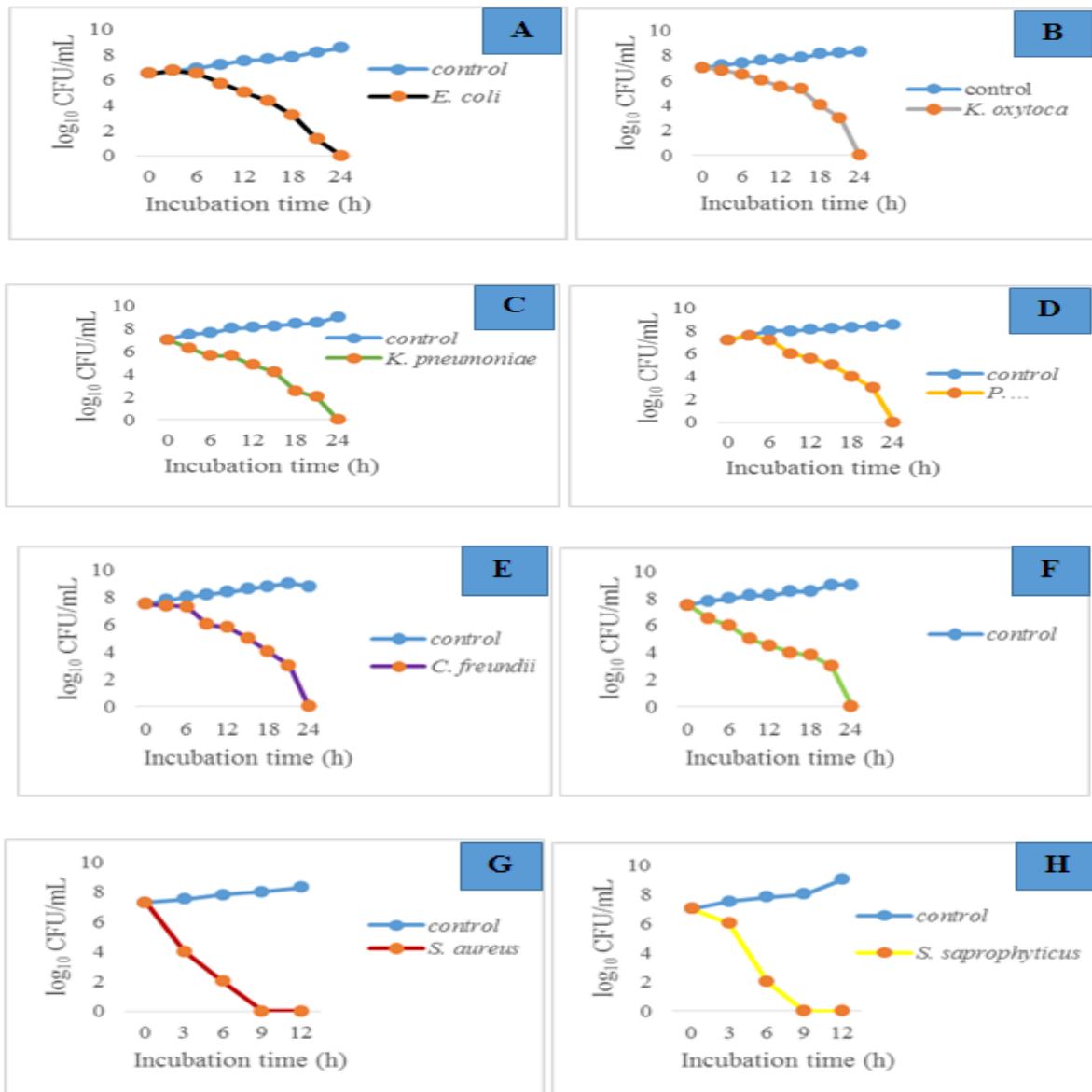


Figure 1: Antibiotics susceptibility against *Proteus mirabilis* isolated in women UTIs. A: presence of zone inhibition around the antibiotic (Imipenem) disc witch show the effect of this antibiotic against *Proteus mirabilis*. B: there are nothing around the antibiotic (Cefotaxime) disc then it show that *Proteus mirabilis* are 100% resistant to this antibiotic.



Figure 2: Antibacterial activity of garlic against *Escherichia coli*. AGE, AGE₁ and AGE₂: 50 µl of AGE at 50% (w/v); A: 50 µl of sterile water (DNase and RNase Free).



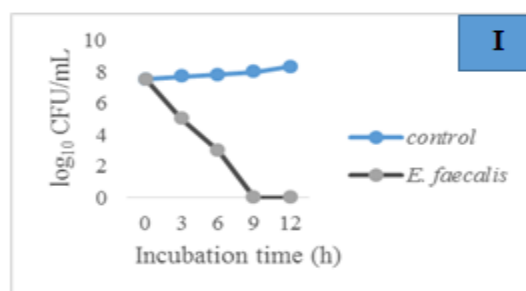


Figure 3: Time bactericidal effect of Togolese garlic on Gram-positive and negative bacteria A: *Escherichia coli*; B: *Klebsiella oxytoca*; C: *Klebsiella pneumoniae*; D: *Proteus mirabilis*; E: *Citrobacter freundii*; F: *Enterobacter aerogenes*; G: *Staphylococcus aureus*; H: *Staphylococcus saprophyticus*; I: *Enterococcus faecalis*.

Table 1: Antibiotics susceptibility of bacteria strains isolated in women urinary tract infection.

	AMC 20/10	CTX 5	CAZ 30	FOX 30	AK 30	CN 500	IMP 10	CIP 5	NA 30	VA 30	K 30	PT 15	L 15	E 15	C 30
<i>E. coli</i>	R	R	S	S	S	S	S	S	S	ND	S	ND	ND	ND	R
<i>K. oxytoca</i>	R	S	R	R	S	S	S	S	R	ND	R	ND	ND	ND	R
<i>K. pneumoniae</i>	R	R	R	R	S	S	S	R	S	ND	R	ND	ND	ND	R
<i>K. ozonae</i>	R	R	R	R	R	S	S	R	S	ND	R	ND	ND	ND	S
<i>C. freundii</i>	R	R	R	S	S	S	S	R	S	ND	S	ND	ND	ND	R
<i>E. aerogenes</i>	R	R	R	S	S	S	S	S	S	ND	R	ND	ND	ND	S
<i>S. aureus</i>	ND	ND	ND	R	ND	S	S	R	R	R	R	R	R	R	R
<i>S. saprophyticus</i>	ND	ND	ND	R	ND	R	R	S	R	R	R	S	S	R	R
<i>E. faecalis</i>	ND	ND	ND	R	ND	R	R	R	R	S	S	R	R	R	S
Susceptible percentage (%)	0	16,67	16,67	33,33	83,33	77,78	77,78	44,44	55,56	33,33	33,33	33,33	33,33	0	33,33

AMC: Amoxicillin/ clavulanic acid, CTX : Cefotaxime, CAZ : Ceftazidime, FOX: Cefoxitin, AK: Amikacin, CN: Gentamycin, IMP: Imipenem, CIP: Ciprofloxacin, NA: Nalidixic acid, VA: Vancomycin, K : Kanamycin, PT: Pefloxacin, L: Lymcomycin, E: Erythromycin, C: Chloramphenicol, R: Resistance, S: Susceptibility, ND: Not Determined,

Table 2: Effect of aqueous garlic extract against strains isolated in women urinary tract infection.

Strains		Aqueous garlic extract 100% (w/v)		
		DZI (mm ±SD)	MIC	MBC
Negative Gram	<i>E. coli</i>	32 ± 4	12,5	12,5
	<i>K. oxytoca</i>	20 ± 3	10	12,5
	<i>K. pneumoniae</i>	25 ± 4	10	12,5
	<i>P. mirabilis</i>	27 ± 3	10	12,5
	<i>C. freundii</i>	23 ± 2	10	12,5
	<i>E. aerogenes</i>	28 ± 2	10	12,5
Positive Gram	<i>S. aureus</i>	45 ± 1	5	5
	<i>S. saprophyticus</i>	38 ± 2	5	10
	<i>E. faecalis</i>	45 ± 1	5	10

DZI (mm ±SD): Diameter of Inhibition (mm ± SD); MIC: Minimum Inhibition Concentration; MBC: Minimum Bactericidal Inhibition.

DISCUSSION

In Table 1, the antibacterial susceptibility testing revealed that bacteria strains are multidrug resistance to standard antibiotics; similar results were previously reported.^[10,12,28] However some bacteria strains exhibited poor sensibility to β lactamins family, aminosid (18.5 %) and fluoroquinolones groups (44.4 %). For women suffering from UTI, conventional management is based on low-dose antibiotic prophylaxis using trimethoprim-sulfamethoxazole (40 mg/200 mg daily), nitrofurantoin (100 mg/day), or cephalexin (250 mg daily).^[11,29] The

use of some antibiotics such as sulfonamide, trimethoprim, quinolones, tetracycline and aminoglycoside was known to increase the health risk.^[30,31,11] Thus, there is an urgent requirement for the development of new drugs constituting an alternative to standard antibiotics, which are not risk for women during pregnancy.

From Table 2, the inhibition zone values showed that the garlic extract had high antibacterial activity against the nine MRB tested. The inhibition diameters range from 20

± 3 to 32 ± 4 mm and MICs range from 10 to 12.5% in Gram-negative bacteria. Gram-positive bacteria exhibited inhibition diameters range from 38 ± 2 to 45 ± 1 mm, and the MIC and MBC values are 5% (w/v). These results are consistent with an earlier study but with less inhibitions diameters zone.^[22,24] The higher potency of Togolese garlic could be related to several factors, including climatic conditions, conferring different properties to garlic due to differences in chemical composition of lipids, proteins, vitamins and carbohydrates that seem to influence antimicrobial activity.^[13]

In addition, Figure 1 show the effect of garlic on the viability of bacterial strains in time. It's demonstrate that the treatment of bacterial strains with garlic extract was able to kill in eight hours (Gram-positive bacteria) to twenty-four hours (Gram-negative bacteria). The bactericidal action of garlic extract appears to be different depending on the bacterial strain. This was probably related to the composition of the cell envelope.^[32,33] Garlic extract interacts with the cell wall and changes the lipid membrane profile, demonstrating the shorter Gram-positive destruction time than the negative.

CONCLUSIONS

In conclusion, the aqueous garlic extract have great potential as antibacterial agent against the nine multidrug resistance bacteria strains examined. Therefore, Togolese garlic may be a promising alternative that could replace antibiotics used in the treatment of urinary tract infections, especially in pregnant women whose use conventional antibiotics that will have repercussions on the health of mother and child. There is a continuous need for the development of new strategies for treatment of UTIs and further investigations are necessary to appreciate *in vivo* effect of garlic for the development of garlic medical use and its promotion.

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