Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2024; 13(1): 322-326
Received: 14-12-2023
Accepted: 21-01-2024

Nandhini V
Department of Microbiology and Biotechnology, Thiagarajar College, Madurai, Tamil Nadu, India

Kaarunya E
Department of Microbiology and Biotechnology, Thiagarajar College, Madurai, Tamil Nadu, India

CM Archana
Department of Microbiology and Biotechnology, Thiagarajar College, Madurai, Tamil Nadu, India

Corresponding Author:
Kaarunya E
Department of Microbiology and Biotechnology, Thiagarajar College, Madurai, Tamil Nadu, India

In-vitro antibacterial activity of Kodai hill garlic (*Allium sativum*) aqueous extract against wound infection pathogens including MRSA

Nandhini V, Kaarunya E and CM Archana

DOI: https://doi.org/10.22271/phyto.2024.v13.i1d.14847

Abstract
The present study attempts to validate the native Indian variety Kodaikanal hill garlic *Allium sativum'* antibacterial efficacy and antioxidant potential against pathogens isolated from wound infections. The agar well diffusion method was used to determine the antibacterial potential of *Allium sativum aqueous extract* against four Gram-negative bacterial strains and one multi drug resistance strain from the wounds. The findings demonstrate that aqueous extract of *Allium sativum* has significant inhibitory effect on MRSA with 42 mm zone formation. Similarly, it was found effective against *E. coli* and *Proteus vulgari*, which gave an inhibition zone of 26 mm and 30 mm respectively at higher dilutions. However, *Pseudomonas aeruginosa* displayed resistance to low dilutions of aqueous extract but inhibition was observed in higher concentration. This study does not undermine the value of antibiotic use but then indicates evidence supporting the traditional use of garlic for wound healing.

Keywords: Garlic aqueous extract, MRSA, antibacterial activity, wound healing

Introduction
Wound infections can be mild, self-healing or severe and life threatening. The most frequent species of microorganisms that cause wound infections are *Acinetobacter* spp., *Pseudomonas* spp., *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Citrobacter*, and anaerobes such as *Clostridium* and *Peptostreptococcus* spp [1, 2]. It has become more challenging to treat wound infections because of the presence of polymicrobial flora, and methicillin-resistant Staphylococcus aureus (MRSA). Antimicrobial resistance, which has become a great threat to public health in the world, is also a major problem in all clinical settings [3]. MRSA is one of the most common strains of wound infections, affecting large areas of the skin that are deeper into the soft tissues, including abscesses, burns, cellulitis, or deep ulcers with infections. According to Ayurveda medicine, garlic is a common ingredient of many medicinal and dietary medicinal formulations as it possesses extensive range of pharmacological action. It is commonly also used as antimicrobial agents in their raw form for the treatment of wounds and injuries and joint pains etc. The natural products are found to be more effective with least side effects as compared to commercial antibiotics so that reason they are used an alternated remedy for treatment of various infections [4].
Garlic also contains essential chemical compounds which are very good for the wound healing process including flavonoids, saponins, alkaloids, and phenolics which are known to have antibiotic principles, essential oils which have antibacterial and antiseptic properties to prevent infection in wounds. As garlic has been reported to possess the active substance allicin which has properties as an antimicrobial, antiseptic, antibiotic, anti-inflammatory and analgesic [5]. The present study aimed to determine *in vitro* antimicrobial activity of garlic aqueous extracts (*Allium sativum*) against isolated MRSA and other wound pathogens.

Materials and Methods
Preparation of aqueous garlic extract
Fresh garlic (*Allium sativum*) was collected from a local market. They were peeled, washed, and blot-dried at room temperature. 25 g of peeled garlic was weighed, washed with 70% ethanol for 2 minutes for surface sterilization, and blot dried. The garlic was then crushed in a sterile mortar and pestle with 10 ml of autoclaved distilled water. The mixture was filtered through Whatman No. 1 filter paper, and the extract collected was considered 100% fresh aqueous extract (AGE) and kept at 4 °C for further analysis.

Phytochemical Screening
The fresh garlic aqueous extract was screened for the presence of secondary metabolites [6]. 20 ml of the extract was measured into different test tubes and concentrated by placing them in a water bath. Tests were carried out for reducing sugars, tannins, flavonoids, alkaloids, and steroids.

Pus sample collection
The pus samples were collected from outpatients at Alpha Labs, Madurai. The skin around the wound was cleansed with sterile, normal saline. A total of 10 swab samples were aseptically collected from the inner surface of the infected area using sterile cotton swabs, immediately immersed in peptone broth, and brought to the microbiology laboratory within 2 hours.

Isolation and identification of bacterial strains
On reception, the swab immersed in peptone broth was then sub cultured on MacConkey Agar and Nutrient Agar (Hi-Media). The plates were incubated at 37 °C for 24 to 48 hours. Lactose fermentation was observed and recorded on a MacConkey agar plate for Methicillin Resistance *Staphylococcus aureus* MRSA, *E. coli*, *Klebsiella pneumoniae*, and non-lactose fermentation were observed for *Pseudomonas aeruginosa*, *Proteus* vulgaris, and *Candida* spp. These bacteria were selected based on their potential to cause skin and wound infections. The characterization of the bacterial isolates was done based on colonial morphology, Gram staining, and a series of secondary biochemical tests such as the catalase test, oxidase test, citrate utilization test, indole test, motility, mannitol, triple sugar iron agar, coagulase test, and urease test. Identification of all source test organisms obtained on MacConkey Agar was carried out according to Bergey's Manual of Systematic Bacteriology [7].

Identification of MRSA
Catalase test [8]
2 ml of hydrogen peroxide solution was added in a test tube, and a sterile swab containing test organisms was put in the hydrogen peroxide solution. The positive results were indicated by immediate bubbling.

Coagulase test [8]
A culture of the test organism was emulsified in a drop of physiological saline and made into a thick suspension. Then a drop of plasma was added to it and mixed gently by rotating. The positive results were indicated by producing clumps within 10 seconds.

Detection of Methicillin Resistance
MRSA was identified based on colonial morphology, gram stain, biochemical responses to catalase, coagulase, Mannitol salt agar test and sensitivity to the second-generation cephalosporin medication cefoxitin (5 µg). After a 24-hour incubation period at 35 °C, the isolates were determined to be methicillin-resistant if there was evidence of growth (>1 colony), sensitive if there was a zone of inhibition over 12 mm, and intermediate if it was found between 11 and 12 mm [9].

Antimicrobial activity
Various dilutions of garlic were used to prepare an aqueous extract, including 25%, 50%, and 75%. The isolated bacterial pathogens were inoculated into the Muller-Hinton agar plates and allowed them to dry in an incubator at 37 °C for 20 minutes [10]. For agar well diffusion assay, the surface of the plates was cut using a micropipette tip borer with a diameter of 6 mm to create uniform wells. Each well was then filled with 100 µl of each dilution and the plates were then incubated at 37 °C for 24 hours. The zone of inhibition diameter was measured in millimetres (mm) [11].

Antioxidant activity
0.1 mM DPPH (1, 1-diphenyl-2, -picryl-hydrazyl) solution was prepared by dissolving 3.94 mg of DPPH in 100 ml of ethanol. Each dilution of fresh aqueous garlic extracts prepared were made up to 40 µl with DMSO and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in a dark condition at room temperature for 20 min. The absorbance was measured at λ max 517 nm [12]. The test results are compared with the ascorbic acid as positive control and ethanol as a blank. The percentage DPPH radicals' inhibition of the test samples is calculated following the equation:

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + sample extract.

Results
The organisms isolated from wound samples were identified to be *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa and* Methicillin Resistance *Staphylococcus aureus* based on morphological and biochemical characteristics using Bergey's Manual of Systematic Bacteriology (Table 1 & 2) (Figure 1).

Table 1: Biochemical test for gram negative organism

Biochemical tests	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*
Gram's stain	-	-	-	-
Motility	Motile	Non-motile	Motile	Motile
Indole test	+	-	-	+
Methyl red	+	-	-	+
Voges-Proskauer	-	+	-	-
Citrate	-	+	+	+
Mannitol	+	+	-	-
TSI	A/A with gas	A/A with gas	K/K	K/A with H_2S
Urease	-	+	-	-

+ = Positive, -- = Negative, A = Acid, `K = Alkali

Table 2: Biochemical test for gram positive organism

Biochemical tests	*Staphylococcus aureus*
Gram's stain	+
Motility	-
Indole test	-
Methyl red	+
Voges-Proskauer	+
Citrate	+
Mannitol	+
TSI	A/A
Urease	+
Catalase test	+
Coagulase test	+
Antibiotic sensitivity	Cefoxitin resistant (12 mm)

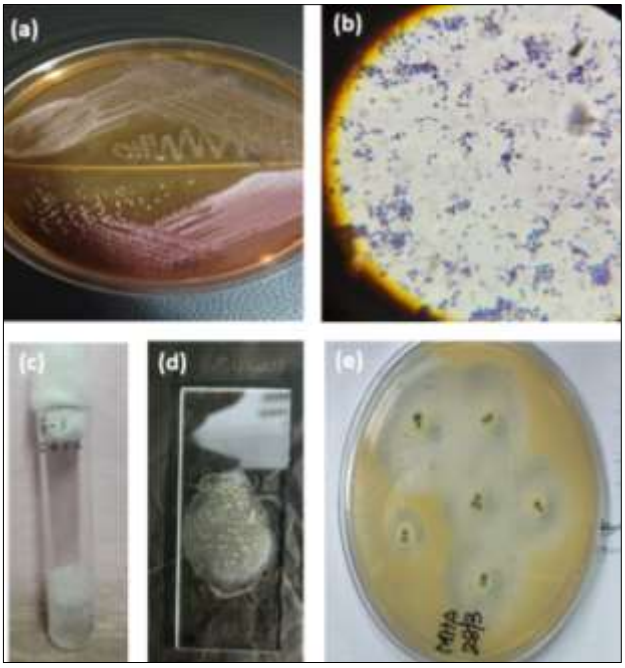

Fig 1: Biochemical tests for specific for MRSA identification
(a) Urease plate (b) Gram's stain (c) catalase test (d) coagulase test (e) Cefoxitin resistant

Phytochemical Screening

The qualitative phytochemical screening of *Allium sativum* aqueous extracts is presented in Table 3. The result indicated the presence of Alkaloids, flavonoids, steroids, phenols, and absence of reducing sugars and tannins.

Table 3: Qualitative phytochemical profile of *A. sativum* aqueous extract

Phytochemicals	Aqueous extract
Alkaloids	++
Flavonoids	++
Reducing Sugar	--
Tannins	--
Steroids	++

+ present; - absent

Antimicrobial Activity

The antimicrobial effectiveness of fresh garlic extract at various dilutions (25%, 50%, and 75%) was assessed by measuring inhibition zones against MRSA and other bacterial pathogens. The results indicated that aqueous garlic extract possessed antibacterial activity against all organisms tested, with different levels of sensitivity. Among all the tested pathogens, aqueous garlic extract exhibited the highest efficacy against MRSA (ZOI - 42mm). In contrast, *Pseudomonas aeruginosa* exhibited resistance to 25 µl concentration (Figure 2a- 2e).

Table 4: Effect of aqueous garlic extract against isolated pus pathogens

Pathogens isolated from wound specimens	**25 µl**	**50 µl**	**75 µl**
MRSA	30 mm	35 mm	42 mm
Escherichia coli	22 mm	24 mm	26 mm
Klebsiella pneumoniae	22 mm	20 mm	26 mm
Pseudomonas aeruginosa	Resistant	15 mm	20 mm
Proteus vulgaris	25 mm	27 mm	30 mm

Fig 2(a): Antibacterial activity of aqueous garlic extract against Methicillin Resistance *Staphylococcus aureus*

Fig 2(b): Antibacterial activity of AGE against *E. coli*

Fig 2(c): Antibacterial activity of AGE against *Klebsiella pneumoniae*

Fig 2(d): Antibacterial activity of AGE against *Pseudomonas aeruginosa*

Fig 2(e): Antibacterial activity of AGE against *Proteus vulgaris*

Antioxidant activity

The results of *Allium sativum* aqueous extract's free radical scavenging abilities in comparison to ascorbic acid was reported in Figure 3. When compared to regular ascorbic acid,

the aqueous garlic extract's DPPH radical scavenging activity was less pronounced but still significantly reduced ROS.

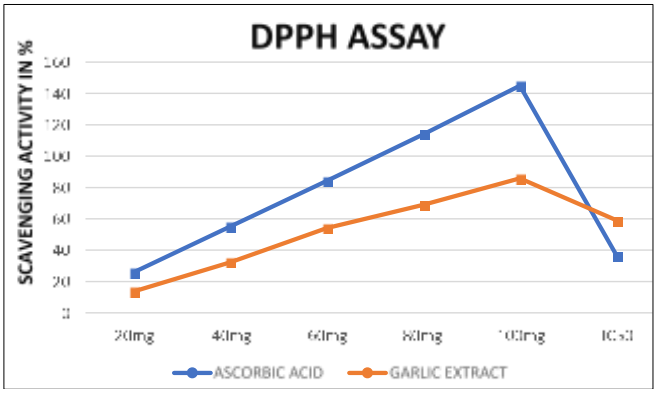

Fig 3: Antioxidant activities of aqueous extract of *A. sativum* as compared to ascorbic acid

Discussions

There are several reports of antibacterial activity of aqueous garlic extract (AGE) against a variety of wound infection causing pathogens [13-17]. Different studies reported that AGE exhibited activity against a large variety of Gram-positive and Gram-positive pathogenic bacteria including MDR strains [18]. A similar trend was reported, where aqueous extract of *Allium sativum* at the minimal concentration of 6.25% successfully inhibited the growth of methicillin-resistant S. aureus MRSA [19]. It has been reported that aqueous extracts of garlic showed antibacterial activity against MRSA [20, 21]. *P. aeruginosa* showed no inhibition against *A. sativum* aqueous extract [22]. Correspondingly, antimicrobial activity of garlic extracts against *P. aeruginosa* found no activity but susceptible to higher concentrations while, growth of *E. coli* was significantly reduced by distilled water extracts of aged garlic [23, 24]. Research has proven that garlic extract can accelerate wound healing and decrease infection risk by activating fibrinogen, an essential factor for wound healing, using Allicin, a compound found in garlic [25]. Although the mechanism by which garlic acts on polymicrobial flora of wound is not fully understood, but has a significant effect on the growth reduction of bacterial species. This may be because of its secondary metabolites particularly allicin, affect bacterial growth by inhibiting their DNA and protein synthesis [26]. Several studies have suggested that garlic contains plentiful phenol, flavonoid, and various sulfur compounds such as S-ally-(L)-cysteine (SAC, hydrophilic) and disulfide (hydrophobic) with high radical scavenging activities [27]. The use of medicinal plants as an alternative could bring about a wide range of therapeutic options, including the prevention of microbial drug resistance [28, 29].

Conclusion

This study has demonstrated the effectiveness of aqueous garlic extract against lacerated wound pathogens and displayed inhibition much greater in MRSA. Due to frequent use of commercial topical antibiotics for the wound infections there is rapid emergence of antibiotic resistance strains. Therefore, research should be extensively focused on the characterization of secondary metabolites, stability, bioavailability, solubility and essentially to understand the precise mechanism of the compounds.

Acknowledgement: The authors acknowledge our college management and department for their support to perform this work successfully.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Percival SL, Cooper RA, Lipsky BA. 11 Antimicrobial interventions for wounds. Microbiology of wounds. 2010;26:293.
2. Forbes BA, Sahm DF, Weissfeld AS, Bailey WR. Diagnostic microbiology. St Louis: Mosby Elsevier, China; c2007.
3. Upreti N, Rayamajhee B, Sherchan SP, Choudhari MK, Banjara MR. Prevalence of methicillin resistant *Staphylococcus aureus*, multidrug resistant and extended spectrum β-lactamase producing gram negative bacilli causing wound infections at a tertiary care hospital of Nepal. Antimicrob Resist Infect Control. 2018;7(1):1-10.
4. Kshirsagar MM, Dodamani AS, Karibasappa GN, Vishwakarma PK, Vathar JB, Sonawane KR, *et al*. Antibacterial activity of garlic extract on cariogenic bacteria: An *in vitro* study. Ayu. 2018;39(3):165.
5. Mustamin R, Taher R, Mallongi A. Efficacy of topical cream of garlic extract (*Allium sativum*) on wound healing in experimental mice using an acute wound modeling: Determination of expression of tumor necrotic factor (TNF-α). Indian J Public Health Res Dev. 2019, 10(10).
6. Sofowora A. Research on medicinal plants and traditional medicine in Africa. J Altern. Complement. Med. 1996;2(3):365-72.
7. Boone DR, Castenholz RW, Garrity GM. Bergey's manual® of systematic bacteriology. Springer Science & Business Media 2001, 2(3).
8. Cheesbrough, Monica. District laboratory practice in tropical countries, part 2. Cambridge university press; c2005.
9. Aboulwafa MM, Aboshanab KM, Haleem Hassouna NA. Virulence characters of some Methicillin resistant *Staphylococcus aureus* isolates. Arch. Clin. Microbiol, 2014, 5(4).
10. Cutler RR, Wilson P. Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*. Br. J Biomed. Sci. 2004;61(2):71-4.
11. Wayne PA. CLSI Performance Standards for Antimicrobial Disk Susceptibility Tests M100; 29th ed. Clinical and Laboratory Standards Institute, 2019, 32.
12. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae) *Food Chem* 2008; 111(4):925–929.
13. Abubakar EM. Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniea and Pseudomonas aeruginosa*. J Med. Plant Res. 2009;3(4):179-85.
14. Abdulrahman D, Daskum A, Abdulrahim K, Dadile A, Amma H. Antibacterial potency of garlic extract against certain skin pathogenic bacteria. Nov. Res. Microbiol. J. 2017;1(1):3-13.

15. Oyawoye OM, Olotu TM, Nzekwe SC, Idowu JA, Abdullahi TA, Babatunde SO, *et al*. Antioxidant potential and antibacterial activities of *Allium cepa* (onion) and *Allium sativum* (garlic) against the multidrug resistance bacteria. Bull Natl Res Cent. 2022;46(1):1-7.
16. Afunwa RA, Okonkwo TC, Egbuna RN, Ikegbune C. Comparative Effects of *Allium sativum* (Garlic) and *Allium porrum* (Leek) on lacerated wound isolates. Open J. Intern. Med. 2022;12(04):184-93.
17. Hemmatpor Z, Kamali J, Mehrabani M, Hashemi SA, Marashi SM, Pahlevan S, *et al*. Study the effect of aqueous extract of garlic (*Allium sativum*) on healing procedure of burn wound on rat. Egypt J Vet Sci. 2020;51(2):181-9.
18. Farrag HA, Hosny AE, Hawas AM, Hagras SA, Helmy OM. Potential efficacy of garlic lock therapy in combating biofilm and catheter-associated infections; experimental studies on an animal model with focus on toxicological aspects. Saudi Pharm J. 2019;27(6):830-40.
19. Magryś A, Olender A, Tchórzewska D. Antibacterial properties of *Allium sativum* L. against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. Arch. Microbiol. 2021;203:2257-68.
20. Al Masaudi SB, Albureikan MO. Antimicrobial activity of garlic juice (*Allium sativum*), honey and garlic-honey mixture on some sensitive and multi resistant microorganism. Life Sci. 2013;10:2429–35.
21. Venâncio PC, Figueroba SR, Nani BD, Ferreira LE, Muniz BV, Del Fiol FD, *et al.* Antimicrobial activity of two garlic species (*Allium sativum* and *A. tuberosum*) against Staphylococci infection. *in vivo* study in rats. Adv. Pharm. Bull. 2017;7(1):115.
22. Abderrahim LA, Taïbi K, Abderrahim NA, Boussaid M, Rios-Navarro C, Ruiz-Saurí A. Euphorbia honey and garlic: Biological activity and burn wound recovery. Burns. 2019; 45(7):1695-706.
23. Saxena G, Sadawarte K, Kaore NM. Antibacterial activity of aqueous extract of garlic (*Allium sativum*) on standard strains. J. Evolution Med. Dent. Sci. 2018;7(19):2320-2322
24. Jang HJ, Lee HJ, Yoon DK, Ji DS, Kim JH, Lee CH. Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents. Food Sci. Biotechnol. 2018;27:219-25.
25. Alhashim M, Lombardo J. Mechanism of action of topical garlic on wound healing. Dermatol. Surg. 2018;44(5):630-4.
26. Feldberg RS, Chang SC, Kotik AN, Nadler M, Neuwirth Z, Sundstrom DC, *et al*. *In vitro* mechanism of inhibition of bacterial cell growth by allicin. Antimicrob Agents Chemother. 1988;32:1763–1768.
27. Bae SE, Cho SY, Won YD, Lee SH, Park HJ. Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment. LWT - Food Sci. Technol. 2014;55(1):397-402.
28. Bhardwaj M, Singh BR, Sinha DK, Kumar V, Prasanna OR, *et al*. Potential of herbal drug and antibiotic combination therapy: A new approach to treat multidrug resistant bacteria. Pharm Anal Acta. 2016;7(11):1-14
29. Reiter J, Levina N, Van der Linden M, Gruhlke M, Martin C, Slusarenko AJ. Diallylthiosulfinate (Allicin), a volatile antimicrobial from garlic (*Allium sativum*), kills human lung pathogenic bacteria, including MDR strains, as a vapor. Molecules. 2017;22(10):1711.