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Comparison Of Antimicrobial Activity Of Various Preparations Of *Allium sativum L.* (Garlic) Against Selected Pathogens

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Abstract: *Allium sativum L.*, also called as Garlic has always remained a dietary item and an age-old remedy with proven antimicrobial efficacy. This study attempts to investigate in comparative terms and test the antimicrobial activity of sun-dried, air-dried, and wet garlic against selected bacterial strains. The zones of inhibition from each garlic sample on Mueller Hinton agar seeded with the respective bacteria were measured by the agar disk diffusion technique. However, there existed clear discrepancies in the antimicrobial activity depending upon the kind of garlic tested and the strain of bacteria involved in the testing. Thus, air-dried garlic was most potent in inhibiting *Staphylococcus aureus*, whereas sun-dried garlic was strongest in its antimicrobial activity against *Escherichia coli.* Least antimicrobial activity against both strains was manifested by the wet garlic, suggesting a drying process that may increase the bioavailability or concentration of bioactive compounds, such as Allicin. Therefore, the results of this study prove that any difference in the processing of garlic significantly affects its antimicrobial activity; hence, the differences ought to be taken into consideration in clinical and food preservation uses. Thus, the natural antimicrobial activity of dried garlic is accentuated here, with considerable implications in pharmaceuticals, nutraceuticals, and food safety.

***Index Terms* – Disk diffusion, zone of inhibition, bioactive compounds, antimicrobial.**

I. Introduction

Allium sativum L. (Garlic) has been valued for its therapeutic value since ancient times, especially in traditional systems such as traditional Chinese medicine and Ayurveda. Throughout history, it has been used to treat a variety of conditions, including gastrointestinal and respiratory illnesses, and skin diseases (Shang et al., 2019). Aside from its antimicrobial actions, garlic is also known to have potential in hormonal balance, inflammation management, cancer prevention, and cardiovascular health (Ried, et al., 2016).

Over the past few years, the mounting problem of antimicrobial resistance in typical pathogens such as *Staphylococcus aureus* and *Escherichia coli* has strengthened the quest for new therapeutic agents. Garlic's dense richness in bioactive components, particularly organosulfur compounds like Allicin (Ankri & Mirelman, 1999), diallyl disulfide, and s-allyl cysteine, have been recognized for their strong antimicrobial properties. These compounds have been shown to possess inhibition activities against a wide range of microorganisms, including drug-resistant organisms (El-Saber Batiha et al., 2020).

The effectiveness of garlic's antimicrobial activity depends on its presentation and processing. For example, aged garlic extracts exhibit higher antioxidant and antibacterial capacities (Sarangi et al., 2024) than fresh garlic due to greater levels of phenolic and flavonoid compounds (Jang et al., 2017). In addition, garlic essential oil, which is high in allyl sulfur compounds, has shown promising antibacterial and antibiofilm actions against several foodborne pathogens, indicating its possible use as a food preservative and safety agent (Harris et al., 2001). In addition, garlic's antimicrobial activity depends on the genotype and conditions under which it is grown. Studies on several Greek garlic genotypes showed considerable variation in their antimicrobial and antioxidant activities, in line with their total phenolic content. These results highlight the significance of genetic and environmental factors in being responsible for the therapeutic potential of garlic (Petropoulos et al., 2018).

II. Materials

Sr. No.	Type	Particular
1.	Samples	Air-dried, Sun-dried, Wet (Fresh) Garlic
2.	Reagents	5.0 % Dimethyl sulfoxide (DMSO)
		Absolute Ethanol
3.	Medium used	Sterilized Mueller Hinton Agar
4.	Glassware	Sterile petri plates
		Sterile test tubes
		Sterile 1.0 ml, 10.0 ml pipettes
		Clean dry beakers
		Clean dry test tubes
		Clean dry pipettes of 1.0 ml and 10.0 ml
		Clean dry measuring cylinder
		Clean dry filtration funnel
		Clean dry glass rod stirrer
5.	Microorganisms Cheesbrough (2006)	*Escherichia coli* culture (18-24 hours old, adjusted to 0.1 Optical density at 530 nm)
		Staphylococcus aureus culture (18-24 hours old, adjusted to 0.1 Optical density at 530 nm)
6.	Miscellaneous	Muslin cloth
		Sterile Whatman filter paper discs
		Sterile cotton swab
		Clean dry forceps
		Spatula
		Analytical weighing balance
		4 °C Refrigerator
		37 °C Incubator
		Magnetic stirrer
		Mortar and pestle
		Autoclave for steam/moist heat sterilization
		Distilled water

III. Methods

A) Garlic Extract Preparation: Garlic that had been sun-dried, air-dried, and wet (fresh) was weighed precisely within 0.75-1.00 grams. After being grounded with a mortar and pestle, the samples were mixed with an 8:2 solvent mixture of Absolute Ethanol and distilled water. Because it acquires both hydrophilic and lipophilic bioactive compounds that, according to Park et al. (2017), support the antifungal activity of plant extracts, this solvent mixture is frequently used during the extraction process. In order to properly extract all bioactive constituents for numerous other phytochemical and antimicrobial studies, the extracts were allowed to cool for 24 hours at about 4 °C (Mohsenipour & Hassanshahian, 2015). Following incubation, 10 ml of 5.0 % Dimethyl Sulfoxide (DMSO) (Jang et al., 2017) was added to condense the active ingredients. The integrity of bioactive species is unaffected by the broad range of organic and inorganic molecules that DMSO can dissolve (Abidullah et al., 2021).

B) Preparation of Culture Plates: Sterile Mueller-Hinton agar (MHA) plates were prepared (HiMedia Laboratories, n.d.) and swabbed with standardized bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus*. This medium is recommended by clinical guidelines due to its consistent composition and optimal diffusion properties, which support accurate measurement of antimicrobial activity using the disk diffusion method (Dewees et al., 1970). To assess the antimicrobial potential of prepared garlic extracts, the agar disk diffusion method was employed, adhering to standardized protocols for assessing antibacterial activity. (European Committee on Antimicrobial Susceptibility Testing [EUCAST], n.d.)

C) **Application of Garlic Extracts:** Sterile Whatman filter paper discs were immersed in the prepared extracts of sun-dried, air-dried, and fresh garlic samples. Aseptically, using sterile forceps, the garlic extracts loaded Whatman filter paper discs were placed on the microbial culture inoculated Mueller Hinton agar surfaces. Sufficient space was kept between the garlic extracts impregnated discs to restrict coinciding of the inhibition zones (Nyuykonge et al., 2021).

D) **Pre-diffusion Step:** The plates were then refrigerated at 4°C for 15 minutes to facilitate the diffusion of the extracts into the agar medium prior to incubation, for elevating the fidelity in recording the inhibition zone (Pongtharangkul & Demirci, 2004).

E) **Incubation:** Maintaining aerobic conditions, the culture plates were incubated for 24 hours at 37°C. This incubation period allows for optimal bacterial growth and interaction with the antimicrobial agents present in the garlic extracts (Abidullah et al., 2021).

F) **Measurement of Inhibition Zones:** After 24 hours incubation at 37°C, the diameters of the clear zones surrounding each disc were measured in millimeters. These zones indicate the extent of bacterial growth inhibition and were compared across the different garlic preparations (Badger et al., 2019). This methodology aligns with established practices in antimicrobial research, where garlic extracts have demonstrated inhibitory effects against both gram-positive and gram-negative organisms (Guo, 2014).

IV. Results

Antibiotic susceptibility testing by Disc Diffusion implicated that all 03 samples of *Allium sativum L.* (Garlic); air-dried, sun-dried, and wet (fresh) sample exhibited different sized zones of clearance/inhibition **(Table 1)** against *Staphylococcus aureus* and *Escherichia coli* after 24 hours of incubation at 37 °C. For *Staphylococcus aureus*, the air-dried garlic marks the highest zone of inhibition (22 mm), while the wet (fresh) sample and sun-dried garlic sample showcased the lesser value of inhibition zone which were 19 mm and 16 mm respectively **(Fig. 1)**. The sequence of the zone of inhibition/clearance is as follows: Air Dried > Wet Sample > Sun Dried. Sun-dried garlic sample demonstrated the largest zone of inhibition of 23 mm, followed by air-dried garlic (19 mm) and wet (fresh) garlic sample (17 mm) for *Escherichia coli* **(Fig. 2)**. The sequence of the zone of inhibition/clearance is as follows: Sun Dried > Air Dried > Wet Sample.

V. Discussion

The research highlights that the antibacterial characteristics of *Allium sativum L.* (Garlic) are governed by its storage and processing criteria. Sun-dried garlic showed the greatest antimicrobial activity against *Escherichia coli*, while air-dried and wet garlic came next. Conversely, air-dried garlic showed the greatest activity against *Staphylococcus aureus*, in contrast to wet (fresh) and sun-dried garlic. These results imply that moisture content and processing conditions play a crucial role in determining the antimicrobial efficacy of garlic. Possible Reasons Behind Variations in Antimicrobial Efficacy are stated as follows:

A) **Allicin Content:** In garlic, Allicin is the prime bioactive compound having antimicrobial potential. Its content could be varied due to the processing and storage conditions of garlic (Ankri & Mirelman, 1999). Sun-dried and air-dried garlic could have higher content of Allicin than the wet one, leading to maximum zones of inhibition against *E. coli* and *S. aureus*, respectively.

B) **Enzyme Activity:** Garlic has enzymes such as Alliinase, which catalyse the conversion of Alliin to Allicin, the active antimicrobial constituent (Shang et al., 2019). The enzyme's activity can be affected by storage and processing conditions, and thus antimicrobial activity will vary.

C) **Moisture Content:** The antimicrobial activity of garlic is maybe regulated by its moisture content. Decreased levels of moisture in dehydrated forms (sun-dried, air-dried) can increase concentrations of active substances, contributing to enhanced antimicrobial activity. These parameters together affect the antimicrobial activity of garlic under various conditions. Having a knowledge of these differences can aid in optimizing garlic processing and storage for its efficient utilization as a natural antimicrobial agent.

VI. Conclusion

The current research offers important information regarding the antimicrobial activity of *Allium sativum L.* (Garlic) treated under varied conditions- sun-dried, air-dried, and wet (fresh) forms against clinically significant bacterial isolates like *Staphylococcus aureus* and *Escherichia coli*. The results obtained with the agar disk diffusion assay unequivocally indicate that the drying process has a great impact on the antimicrobial effectiveness of garlic. Air-dried garlic showed the strongest inhibitory action against *S. aureus*, implying that mild dehydration can improve preservation or concentration of bioactive compounds responsible for antimicrobial activity. Sun-dried garlic, on the other hand, displayed greater antimicrobial effects towards *E. coli*, possibly as a result of increased stability of compounds upon exposure to sunlight. Wet (fresh) garlic, despite common use in culinary and medicinal contexts, exhibited the weakest antimicrobial activity, perhaps as a consequence of reduced concentrations or accelerated degradation of active components in its natural state. Results highlight that the way in which *Allium sativum L.* is used considerably modifies its bioactivity, with dried preparations providing improved antimicrobial activity. This also highlights the significance of processing methods in the optimization of the therapeutic value of natural plant-derived antimicrobials. The proven effectiveness of dried garlic proposes beneficial uses in natural food preservation, complementary medicine, and design of herbal antimicrobial products. Future studies should be directed toward quantitative profiling of the major bioactive components in each garlic variety and their stability, mode of action, and their possible synergistic actions with standard antibiotics. Thus, *Allium sativum L.* is a worthwhile contender in the quest for sustainable and inexpensive antimicrobial agents.

Figures and Tables

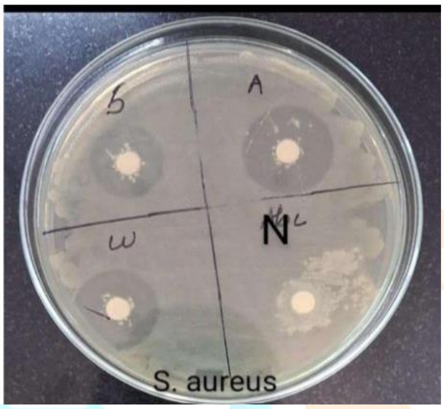

Fig. 1. Zones of inhibition against *Staphylococcus aureus*

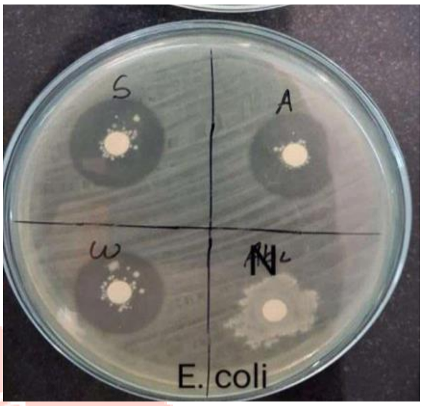

Fig. 2. Zones of inhibition against *Escherichia coli*

(A: Air-dried Garlic sample, S: Sun-dried Garlic sample, W: Wet (fresh) Garlic sample, N: Negative control-disc without any sample)

Table 1. Antimicrobial Susceptibility Testing by Agar Disc Diffusion Method: Zones of inhibition in mm

Microorganism tested	Air-Dried Garlic Sample	Sun-Dried Garlic Sample	Wet (fresh) Garlic Sample
Staphylococcus aureus	22	16	19
Escherichia coli	19	23	17

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