Antibacterial Effect of Garlic (*Allium Sativum*)

On *Staphylococcus Aureus*: An In Vitro Study

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**Abstract**—The methanol and aqueous suspensions of the dried *Allium sativum* (Liliaceae) bulbs extract was screened for its antimicrobial activity using the agar-well diffusion method. It is tested against Gram-positive bacteria (*Staphylococcus aureus*). The suspensions were tested at concentrations of 1, 10, 100 and 1000 μg/ml. All suspensions showed an inhibitory effect against tested bacteria. The highest zone of inhibition was estimated with the highest concentration of aqueous suspension (48 mm) followed by the highest concentration of the methanolic suspensions (1000 μg/ml) which reached to 34 mm. The other concentrations either methanolic or aqueous showed various inhibitory effect on the tested bacteria.

**Keywords**—Antimicrobial activity, traditional herbal, *Allium sativum*, *Staphylococcus aureus*

**I. INTRODUCTION**

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [1]. Garlic (*Allium sativum*) has traditional dietary and medicinal applications as an anti-infective agent [2], [3].

In vitro evidence of the antimicrobial activity of fresh and freeze-dried garlic extracts against many bacteria [4], [5], fungi [6], and viruses [7] supports these applications. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [8].

Recently, medicinal plants have become the focus of intense study regarding their conservation and potential pharmacological effects. Indeed, the search for new pharmacologically active agents, through the screening of natural sources such as microbial fermentations and plant extracts, has led to the discovery of many clinically useful drugs that now play major roles in the treatment of human diseases [9], [10].

About 80% of individuals from developed countries use traditional medicine, which has compound derived from medicinal plants.

Therefore such plants should be investigated to understand their properties, safety and efficacy and for a search of new potent antimicrobial compounds and fractions [11].

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain [12]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs [13]. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms [14].

**II. MATERIAL AND METHODS**

**A. Preparations of plant materials**

Garlic (*Allium sativum*) used in the present study was purchased from the local market. Bulbs were peeled and washed from the foreign particles. A Fifty grams of the bulbs were squeezed. The squeezed sample was sucked at methanol for 8hs with 10 minutes interval shaking.

The extraction was filtered using muslin cloth and then Whatman no. 1 filter paper. The filtrate was evaporated at 45°C to dryness and the dried substance was kept in sterile bottle under refrigerated condition until use.

The dry weight of the extract was obtained by allowing the solvent to evaporate and was used to determine concentration in mg/ml methodology based on [15]. Four concentrations of each extract (1, 10, 100, and 1000 mg/ml) were prepared by resuspending the dried extract in methanol separately. Other same concentrations were resuspended in sterile distilled water (aqueous).

**B. Tested microorganisms**

The test microorganisms (*Staphylococcus aureus*) used in this study was obtained from the Misurata Central Hospital Laboratory. The bacterial isolate was first subcultured in a nutrient broth (Oxoid) and incubated at 37°C for 18 h.

**C. Antimicrobial activity**

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by [16]. Cup-plate method was used to test the antibacterial activities of the aqueous and methanolic *Allium sativum* against the test bacteria isolates.

Eighteen hour broth cultures were diluted appropriately using McFarland scale (0.5 McFarland which is about 10⁶ cfu/ml). The molten sterile nutrient agar (20 ml) was poured into sterile petri dish and allowed to set.

The sterile nutrient agar plates were flooded with 0.2 ml of the standardized inoculum. A sterile cork borer (No. 6) was
used to bore equidistant cups into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. Approximately 50 μl of the crude extract at different concentrations (1 mg/ml, 10 mg/ml, 100 mg/ml and 1000 mg/ml) were introduced into the wells.

A control was prepared by putting 50 μl of freshly prepared sterile distilled water in one of bored hole at the plates containing aqueous suspensions where 50 μl of methanol use at the plates containing methanolic suspensions. One hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 18 h. The zones of inhibition were then measured in millimeter. The above method was carried out in triplicates and the mean of the triplicate results were taken.

D. Preparation of Inoculum

About 18 hour broth culture of the test bacteria isolate was suspended into sterile nutrient broth. It was standardized according to National Committee for Clinical Laboratory Standards [17] by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0 × 10⁷ cfu/ml.

III. RESULTS AND DISCUSSION

The resuspension extract aqueous, and methanolic of Allium sativum and (bulbs) was investigated for their antimicrobial activity against Staphylococcus aureus. The “hole plate” diffusion method was used in testing various concentrations of this extract. The results depicted in Table 1 indicate that the high concentrations of Allium sativum aqueous extract (1000 mg/ml) used in this study had the highest inhibitory effects (48mm) against the tested microorganisms. However, this extract showed inhibition action of 19 mm even at minimal concentration (1 mg/ml) used in this study. The other concentrations of the aqueous phase (10 and 100 mg/ml) gave an inhibition zones of 23 and 27mm, respectively.

Methanolic extract of Allium sativum at all concentrations shows an inhibitory effect against the Staphylococcus aureus. The highest inhibition zone obtained was 34 mm with the concentration of 10 mg/ml shown. The methanolic extract showed lower action compared to the aqueous extract (resuspension) as antimicrobial agents. This may be due to little diffusion properties of the extract in the agar or because fresh plants contain active substances which may be affected or attributed by the used solvent.

Reference [18] reported that 100 mg/ml of the garlic aqueous extract shows an inhibition zone of 19.3mm on Staphylococcus aureus, also reported that garlic methanol extract at concentration of 100mg/ml shows an inhibition zone of 11mm on the same bacteria. However the inhibitory zone of the same concentration at this study showed was higher effect (27 mm) comparing to the mentioned study. This is may be due to the species difference or the garlic difference in different biologic condition. [19] reported that S. aureus (NSA1) was most susceptible to the active principles present in garlic, which had a zone of growth inhibition diameter of 28 mm in water, 23, 24 and 32 mm in chloroform, ethanol and metronidazole respectively. Such results were similar from data reported by Vuddhakul [20] who observed that garlic extracts inhibited the growth of Staphylococcus aureus. In contrast [21]. Reported that crude extracts of garlic did not exhibit any in vitro inhibition on the growth of test organisms including Staphylococcus spp.

The large sizes of zones of growth inhibition produced by the garlic extracts against the tested bacteria indicated the potency of the active principles in garlic.

The study focused on the garlic antibacterial activity on S. aureus has shown that dilute solutions of garlic can completely inhibit the growth of S. aureus at the concentration of more than 7.50 mg/ml. This could be due to the action of biological active ingredient of allicin which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action [22].

Using the same protocol garlic has a bactericidal effect at the lower concentration of 30.00 mg/ml for clinical isolate of S. aureus. However, this concentration level may vary as different authors have stated; for instance 160 mg/ml was observed by [23].

IV. CONCLUSION

It is concluded from this study that Allium sativum extract has antimicrobial activity against Staphylococcus aureus. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

REFERENCES


