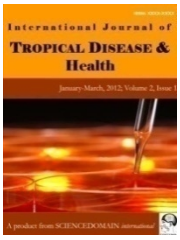

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Antibacterial and Antifungal Activity of Methanolic Leaf Extract of *Allium sativum* on Selected Pathogenic Strains

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSO designed the study, performed the statistical analysis and wrote the protocol, authors JOS and MDA managed the literature searches and wrote the first draft of the manuscript. Authors JOS, EO and MOS managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Allium sativum plants contain chemicals which can inhibit the growth of microorganisms and thus make them suitable to be used in different medicines. The antibacterial and antifungal activities of *Allium sativum* methanolic extracts were investigated using standard analytical techniques and modern micro plate-based antibacterial assays

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techniques. The antibacterial of methanolic extract of *Allium sativum* were used on different bacteria strains which include *Escherichia coli, Pseudomonas aeruginosa, Protea spp., Salmonella typhi and Staphylococcus aureus.* The antifungal observation of *Allium sativum* was found to be active against *Aspergilus flavus, Aspergilus niger, Aspergilus fumigatus, Penicillium spp* compared to Funbact-A (control). The susceptibility test showed that *Allium sativum* is more active against *Aspergilus fumigatus.* The *Allium sativum* had a higher zone of inhibition compared to Ciproflaxin (control) on the different bacteria strains. *Aspergillus fumigatus* and *Aspergillus niger* differs significantly in their degree of inhibition of methanolic extract of *Allium sativum.* The findings support the use of *Allium sativum* extract in treating bacterial infections and Aspergillosis.

Keywords: Allium sativum; antibacterial; antifungal; ciproflaxin; zone of inhibition.

1. INTRODUCTION

Traditional medicine is the most ancient method of curing diseases and it has been said that plants are the very first and only true medicine ever used [1]. Garlic is an economically important plant which belongs to the family Liliaceae, botanical name "*Allium sativum*". Today there is rapidly increasing world-wide interest in *Allium sativum*, and the number of scientific studies performed every year is increasing exponentially [2]. These studies have supported the idea that the regular consumption of garlic can reduce blood pressure and blood cholesterol levels [3] act as an inhibitor to the overgrowth of pathogenic organisms in the body, such as *Candida albicans*. It is also used as a worm medicine, and has a number of other beneficial effects [4]. The bulbs of Garlic are used as condiments and its juice is medicinally very useful [5]. *Allium sativum* has been throughout history for both culinary and medicinal purposes [6]. *Allium sativum* contains more than 100 biologically useful chemicals, substances such as allin, allicin, s-ally cysteine, dially sulphide. Garlic is one of the edible plants which have generated a lot of interest in human history as a medical panacea. It has been used medically for at least 3,000 years, but until recently, its benefits were considered little more than folklore. Many plants are used for the treatment of different diseases and many possess antimicrobial activities [7,8]. Malesh and Satish determined the antimicrobial activity of some important medicinal plant against plant and human pathogen [9]. However, in 1985, Ramesh [10] et al., established that plant extracts are used as medicinal therapies in this modern age. Moreover, *Allium sativum* extracts exhibited activity against both gram negative (*E. coli, Salmonella spp and Citrobacter enterobacter, Pseudomonas spp, Klebsiella spp*) and gram positive bacteria (*Staphylococcus aureus, S. Pneumonia,* Group A streptococcus and *Bacillus anthrax*) all of which are cause of morbidity worldwide [4]. There is extensive literature on the antibacterial effects of fresh *Allium sativum* juice, lyophilized powders, steam distilled oil and other commercial preparations of *Allium sativum* [11]. The present investigation was undertaken to assess the effectiveness of *Allium sativum* on some selected fungal and bacterial spp.

2. MATERIALS AND METHODS

2.1 Test Organisms

Bacterial and Fungal strains were collected from the Veterinary research institute (VOM), Jos, Plateau State, Nigeria. Fungal strains used include: *Aspergilus flavus, Aspergilus niger, Aspergilus fumigatus* and *Penicillium spp.* Bacterial strain such as *Staphylococcus aureus,*

Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli and Proteus species were used. All the cultures were tested for purity. These strains were then inoculated into their various culture media in other to increase their lifespan. This was then kept in the fridge at a temperature of about 10-15ºC (degree Celsius).

2.2 Collection and Identification of Samples

Allium sativa bulb (garlic) was obtained from Jos terminus market, Plateau State Capital in Nigeria. The samples of *Allium sativum* plants were identified by Mr. M.C. Chomin, H.O.D Forest technology Federal College of Forestry, Jos. *Allium sativum* was found to belong to the family of Liliaceae.

2.3 Methanol Extraction of *Allium sativum*

The bulb of *Allium sativa* was peeled, washed and dried at room temperature (37ºC), for three weeks. It was then ground into fine powder using the warring commercial blender. The powdered bulb of *Allium sativa* was weighed using electric balance and 30g of *Allium sativa* was subjected to methanolic extraction using a soxhlet apparatus. The sample was packed into the extraction column, mounted on a 250ml conical flask half filled with methanol, placed on a heating mantle at a temperature of 95ºC, and supported with a retort stand. The extraction process took two hours.

2.4 Concentration of the Methanolic Extract

The crude methanolic extracts obtained were concentrated by evaporation using a rotary evaporator at 4000rev/min at 85ºC and further concentrated by evaporation on a water bath at 80ºC for 1h then stored in sample bottle and the percentage yield was determined as follows:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Weight of the dried plant sample used}} \times 100$$

2.5 Preparation of Crude Extract

The extract was reconstituted by dilution (in sterile distilled water) to various concentrations before using for treatment [12].

2.6 Determination of Antifungal Susceptibility of Methanolic *Allium sativum* Extracts

3.0g of methanolic *Allium sativum* extracts was dissolved in 20ml of distilled water to obtain a concentration of 150mg/ml. 1gm of Funbact-A (reference drug) was dissolved in methanol and made up to 10ml. Five plates in duplicates were seeded with test organisms using a cork borer, three wells were made on each plates, 0.1ml of methanolic *Allium sativum* extracts and the reference drug were introduced aseptically into the wells. The plates were left standing on the work bench for 30minutes to allow for pre-diffusion times incubated at room temperature for 48 hours and the zone of inhibition were measured.

2.7 Determination of Antibacterial Susceptibility of Methanolic *Allium sativum* Extracts

This was carried out using the agar-gel dilution method as described by Osadebe and Ukwueze [13]. In this method, both culture of the test isolates (0.1ml) containing 1 x 10^5 cells/ml of organism was aseptically inoculated by spreading evenly onto the surface of the plates using a bent sterile glass rod. Five wells (12 mm diameter) were then made in the plates using sterile cork borer. The fourth and fifth wells served as negative and positive controls respectively. The methanol served as the negative control, ciproflaxin served as positive control. The bottom of the wells 1-4 was sealed with one drop of the sterile nutrient agar to prevent diffusion of methanolic *Allium sativum* extract under the agar. Fixed volumes (0.1ml) of methanolic *Allium sativum* extracts were transferred into the wells 1-4 using a sterile Pasteur pipette. The control wells were filled with 0.1ml of methanol and ciprofloxacin. The plates were allowed on the bench for 40 min for pre-diffusion of methanolic extract of *Allium sativum* and then incubated at 37ºC for 24hr. Antibacterial activity of methanolic extract of *Allium sativum* were determined by measurement of the resulting zone of inhibition (mm) against each test bacteria using a ruler. The experiment was carried out in triplicates and the mean values of the results were taken as antibacterial activity.

3. RESULTS

3.1 Determination of Antifungal Susceptibility of Methanolic Extracts of *Allium sativum*

The result of each test carried out on different fungal strain with the use of the methanolic extract of *Allium sativum* and Funbact as reference drug (control) can be seen in Table 1 below.

Table 1. Sensitivity test of methanolic extracts of *Allium sativum* on fungal strains (mg/ml)

Test organisms	Control (mg/ml)	Methanolic extract of *Allium sativum* (mg/ml)
Aspergilus fumigatus	14.67±0.58[a]	7.87±0.32[b]
Aspergilus niger	3.60±0.00	2.80±0.20
Aspergilus flavus	3.20±0.00	2.42±1.01
Penicillium spp.	3.33±0.42[a]	2.42±1.01[b]

Results are presented as mean±SD. Values carrying superscripts different from the control for each parameter across the column are significantly different (p<0.05)

From the table above *Aspergillus fumigatus* and *Aspergillus niger* differs significantly due to its highest zone of inhibition of methanolic extract of *Allium sativum.*

3.2 Determination of Antibacterial Susceptibility of Methanolic Extracts of *Allium sativum*

The result of each test carried out on different bacteria strain with the use of the methanolic extract of *Allium sativum* and Ciprofloxacin as reference drug (control) can be seen in Table 2 below.

Table 2. Result on the zone of inhibition of methanolic extract of *Allium sativum* using different bacteria strains

Bacteria strains	Diameter of zone of inhibition (mm)	
	Control (Ciprofloxacin)	*Allium sativum* extract
Escherichia coli	23.0±0.82	27.0±0.29
Salmonella typhi	24.0±0.00	28.0±0.00
Staphylococcus aureus	22.0±0.82	27.0±0.29
Pseudomonas aeruginosa	21.0±0.29	26.0±0.00
Proteus species.	24.0±0.82	27.0±0.29

Values are Mean±S.E.M for five determinations. Diameter of Cork borer=12mm

From the result in the Table 2, it was observed that the methanolic extract of *Allium sativum* appears to have higher zone of inhibition compared to that of control (ciprofloxacin).

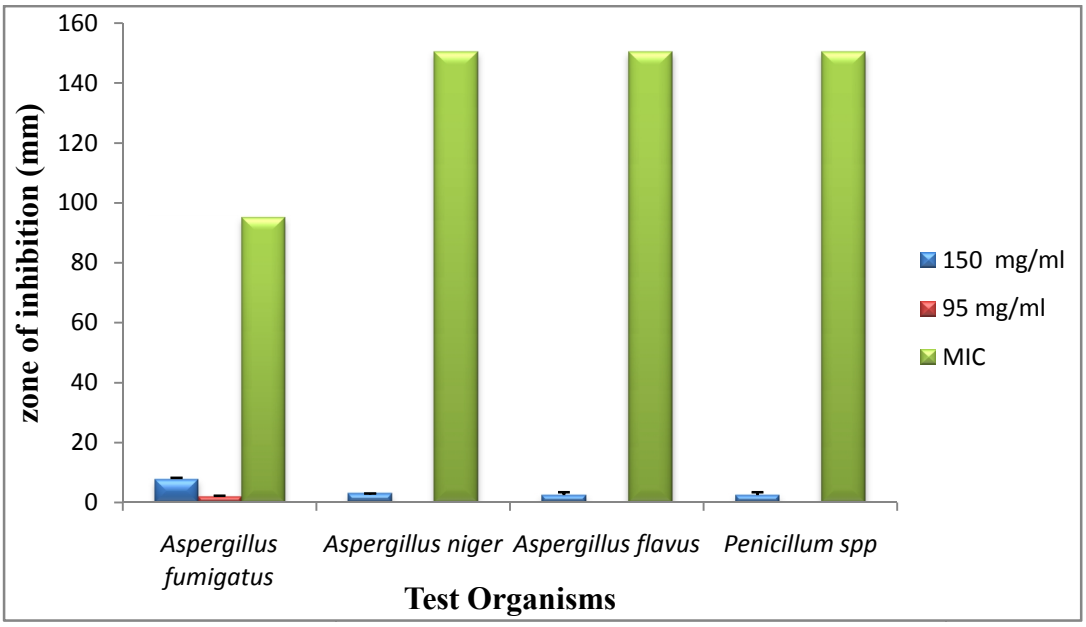

Fig. 1. Minimum Inhibitory Concentration (MIC) of *Allium sativum* on fungal strains at different concentrations

From the Fig. 1 above, *A. flavus*, *A. niger*, *A. flavus* and *P. spp* were inhibited the highest at a concentration of (95 mg/ml) of *Allium sativum.*

4. DISCUSSION

The susceptibilities of the test organisms to plant extracts as reported in this study (Table 1) shows that *Aspergillus fumigatus* and *Aspergillus niger* differs significantly due to its highest zone of inhibition of methanolic extract of *Allium sativum* which is a potent antibiotic and can be particularly effective against certain fungal infections [14]. The minimum inhibitory concentration (MIC) of the fungal strain to plant extracts as reported in this study (Fig 1.) shows that *Aspergillus fumigatus* was inhibited at the lowest concentration (95mg/ml) of the *Allium sativum*, this can be interpreted as the extract having biostatic effect on the organism

[15] *Aspergillus niger* was inhibited at the highest concentration (150mg/ml) of *Allium sativum*. This implies that *Allium sativum* may be very useful in treatment of Aspergillosis caused by *Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger* [16] and Otomycosis (ear infection caused by *A. fumigatus*) Otomycosis can cause pain, temporary hearing loss, and in severe cases, damage to the ear canal and tympanic membrane. However, *Aspergillus flavus* was inhibited at the highest concentration (150mg/ml) of *Allium sativum* and *Penicillium spp* was inhibited at the highest concentration (150mg/ml) of *Allium sativum* (Fig. 1).

The antibacterial activity of methanolic extracts of *Allium sativum* (Table 2) showed potent inhibition on the bacteria strains used which includes *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Proteus species* and the control (Ciprofloxacin). Our findings supported the use of methanolic extracts *of Allium sativum* in herbal medicine [17]. Also, the antibacterial potential of *Allium sativum* supported its use as an antiseptic after birth [18]. It could also be seen that the methanolic extract of *Allium sativum is* active against *Pseudomonas aeruginosa* thereby justifying its use for wound healing. *Escherichia coli* are known to cause diarrhoea and so the activity of methanolic extract of *Allium sativum* from this study against these bacteria justifies its folkloric use in treatment of diarrhoea and urinary tract infection like gonorrhea.

5. CONCLUSION

In light of the result of this present study, the plant extract investigated *Allium sativum* is potent against the test organisms; it has activity against fungal strains, gram-positive and gram-negative bacteria and can be utilized in the production of medicinal portions in situation where conventional drugs are either unavailable or too expensive to purchase to treat various ailments. Antifungal actions of *Allium sativum* are attributed to the biochemical composition, and the quantity of these biochemical compositions per gram of these plant materials.

CONSENT

All authors declare that no consent was obtained for this study.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gill. Ethanomedical use of plants in Nigeria. University of Benin press, Nigeria; 1992.
2. Borelli Ff, Capasso R, Izzo AA. Garlic (*Allium sativum* L.): Adverse effects and drug interactions in humans. MolNutr Food Res 2007;51(11):1386-97.
3. Ackerman RT, Mulrow CD, Raimirez G, Gardner CD, Morbidoni L, Lawrence VA. Garlic shows promise for improve some cardiovascular risk factors. Archives of Internal Medicine. 2001;7(6):696-700.
4. Breithaupt-Grogler K, Ling M, Boudoulus H, Belz. Protective effect of chronic garlic intake on elastic properties of aorta in the elderly. Circulation. 1997;96(8):2649-55.
5. Agarwal SB. Unified botany. Shivalal Agarwal & Company, Agra. P. 1999;167-215.
6. Block E. Garlic and other allium: *The* lore and the science. Royal Society of Chemistry; 2010. ISBN 0854041907.
7. Arora D, Kaur J Antimicrobial activity of spices. Int. J. Antimicrob. Agent. 1999;12:257-262.
8. Cavallito CJ, Bailey JH, Buck J. Allicin, the antibacterial principle of *Allium sativum*. III. Its precursor and “essential oil” of garlic. J. Am. Chem. Soc. 1945;67:1032.
9. Malesh B, Satish S. Antimicrobial Activity of some important medicinal plant against plant and human pathogen. World Journal of Agriculture Sciences. 2008;4(5):839-843.
10. Ramesh A, Hyma B. Traditional Indian medicine in practice in an Indian metropolitan city. In: Geographical aspects of wealth and disease in India. Ed: Akhtar R and ATA Learmouth concept publishing company, New Delhi. 1985;361.
11. Fenwick GR, Hanley AB. The genius Allium-Part 1. Crit. Rev Food Sci Nutr. 1985;22:199-271.
12. Akujobi C, Anyanwu BN, Onyeze C, Ibekwe VI. Antibacterial activities and preliminary phytochemical screening of four medical plants. J. Appl Sci. 2004;7(3):4328-4338.
13. Osadebe PO, Ukwueze. Bio-Research. 2004;2(1):18-23.
14. Bolton S, Null G, Troetel WM. The medical uses of garlic-fact and fiction. Am Pharm. 1982;22:40-388.
15. Presus HG, Echard B, Enig M, Briik I, Elliot TB. Minimum inhibitory concentrations of herbal essential oils and mono laurin for gram-positive and gram-negative bacteria. Molecular Cell Biochemistry. 2005;272:29-34.
16. Klich MA. *Aspergillus flavus*: The major producer of aflatoxin. Molecular Plant Pathology. 2007;8(6):713-22.
17. Adewusi HA. The African star apple *Chrysophyllum albidum* indigenous knowledge from Ibadan, South western Nigeria. In proceedings of a National workshop on the potentials of the star apple in Nigeria (eds). 1997;25-33.
18. Hile AG, Shan Z, Zhang SZ, Block E. Aversion of European starlings (*Sturnus vulgaris*) to garlic oil treated granules: Garlic oil as an avian repellent. Garlic oil analysis by nuclear magnetic resonance spectroscopy. Journal of Agricultural and Food Chemistry. 2004;52:21926.

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