

Original Research Article**ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF
Allium sativum ON SELECTED PATHOGENIC STRAINS****Abstract**

Allium sativum plants contain chemicals which can inhibit the growth of microorganisms and these 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 techniques. The antibacterial of methanolic extract of *Allium sativum* were used on different bacteria strains which include *E.coli*, *Ps. Augroginosa*, *protea spp.*, *Samonella typhi* and *S aureus*. The antifungal observation of *Allium sativum* was found to be active against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium spp* compared to Funbact-A (control). The susceptibility test showed that *Allium sativum* is more active against *Aspergillus fumigates*. The *Allium sativum* had the highest zone of inhibition compared to Ciproflaxin (control) on the different bacteria strains. The findings support the use of *Allium sativum* extract in treating bacterial infections and Aspergillosis.

Keywords: *Allium sativum*, antibacterial, antifungal, Ciproflaxin, zone of inhibition.

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24 **1.0 Introduction.**

25 Traditional medicine is the most ancient method of curing diseases and it has been said that
 26 plants are the very first and only true medicine ever used ^[1]. Garlic is an economically important
 27 plant which belongs to the family Liliaceae, botanical name “*Allium sativum*”. Today there is
 28 rapidly increasing world-wide interest in *Allium sativum*, and the number of scientific studies
 29 performed every year is increasing exponentially ^[2]. These studies have supported the idea that
 30 the regular consumption of garlic can reduce blood pressure, blood cholesterol levels ^[3] act as an
 31 inhibitor to the overgrowth of pathogenic organisms in the body, such as *Candida albicans*, used
 32 as a worm medicine, and have a number of other beneficial effects ^[4]. The bulbs of Garlic are
 33 used as condiments and its juice is medicinally very useful ^[5]. *Allium sativum* has been
 34 throughout history for both culinary and medicinal purposes ^[6]. *Allium sativum* contains more
 35 than 100 biologically useful chemicals, substances such as allin, allicin, s-ally cysteine, diallyl
 36 sulphide. Garlic is one of the edible plants which have generated a lot of interest in human
 37 history as a medical panacea. It has been used medically for at least 3,000 years, but until
 38 recently, its benefits were considered little more than folklore. The use of plants in medicine
 39 goes as far back as thousands of years and still continues today ^[7,8]. Many plants are used for the
 40 treatment of different diseases and many possess antimicrobial activities ^[7]. Malesh and Satish
 41 determined the antimicrobial activity of some important medicinal plant against plant and human
 42 pathogen ^[9]. However, in 1985, Ramesh^[10] *et al.*, established that plant extracts are used as
 43 medicinal therapies in this modern age. Moreover, *Allium sativum* extracts exhibited activity

against both gram negative (*E. coli*, *Salmonella spp* and *Citrobacter enterobacter*, *Pseudomonas kilabsella*) 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, methanolic and alcoholic extracts, lyophilized powders, steam distilled oil and other commercial preparations of *Allium sativum*. Fenwick and Hanely (1985) understood a thorough review of the antibacterial effects of *Allium sativum* and other Allium vegetables up to mid-1984 ^[11]. The present investigation was undertaken to assess the effectiveness of *Allium sativum* on some selected fungal and bacterial spp.

2.0 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: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus 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 order to increase their lifespan. This was then kept in the fridge at a temperature of about 10-15°C (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

66 **2.3 Methanol Extraction of *Allium sativum***

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

74 ***Concentration of the methanolic extract:***

75 The crude methanolic extracts obtained were concentrated by evaporation using a rotary
 76 evaporator at 4000rev/min at 85°C and further concentrated by evaporation on a water bath at
 77 80°C for 1h then stored in sample bottle and the percentage yield was determined as follows:

$$78 \text{ Percentage yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Weight of the dried plant sample used}} \times 100$$

79

80 ***Preparation of Crude Extract***

81 The extract was reconstituted by dilution (in sterile distilled water) to various concentrations as
 82 described by Akujobi et al.(2004) before using for treatment.

83 ***Preparation of Sabouraud Dextrose agar. (SDA).***

84 6.0g SDA was weighed and dissolved in 100ml of deionized water. It was allowed to boil on hot
 85 plate for proper dissolution, it was then sterilized at 121°C in an autoclave for 15 minutes.

86 ***Determination of antifungal susceptibility of methanolic Allium sativum extracts.***

87 3.0g of methanolic *Allium sativum* extracts was dissolved in 20ml of distilled water to obtain a
 88 concentration of 150mg/ml. 1gm of Funbact-A (reference drug) was dissolved in methanol and
 89 made up to 10ml. Five plates in duplicates were seeded with test organisms using a cork borer,
 90 three wells were made on each plates, 0.1ml of methanolic *Allium sativum* extracts and the
 91 reference drug were introduced aseptically into the wells. The plates were left standing on the
 92 work bench for 30 minutes to allow for pre-diffusion times incubated at room temperature for 48
 93 hours and the zone of inhibition were measured.

94 ***Determination of antibacterial susceptibility of methanolic Allium sativum extracts.***

95 This was carried out using the agar-gel dilution method as described by Osadebe and Ukwueze
 96 (2004). In this method, both culture of the test isolates (0.1ml) containing 1×10^5 cells/ml.
 97 Organism was aseptically inoculated by spreading evenly onto the surface of the plates using a
 98 bent sterile glass rod. Five wells (12 mm diameter) were then made in the plates using sterile
 99 cork borer. The fourth and fifth wells served as negative and positive controls respectively. The
 100 methanol served as the negative control, ciproflaxin served as positive control. The bottom of the
 101 wells 1-4 was sealed with one drop of the sterile nutrient agar to prevent diffusion of methanolic
 102 *Allium sativum* extract under the agar. Fixed volumes (0.1ml) of methanolic *Allium sativum*
 103 extracts were transferred into the wells 1-4 using a sterile Pasteur pipette. The control wells were
 104 filled with 0.1ml of methanol and ciprofloxacin. The plates were allowed on the bench for 40
 105 min for pre-diffusion of methanolic extract of *Allium sativum* and then incubated at 37°C for
 106 24hr. Antibacterial activity of methanolic extract of *Allium sativum* were determined by
 107 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.

Results

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.

Test organisms	Control	methanolic extract of <i>Allium sativum</i>
<i>Aspergillus fumigatus</i>	14.67 + 0.58 ^a	7.87+ 0.32 ^b
<i>Aspergillus niger</i>	3.60 + 0.00	2.80 + 0.20
<i>Aspergillus flavus</i>	3.20 + 0.00	2.42 + 1.01
<i>Penicillium spp.</i>	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).

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)	<i>Allium sativum</i> extract
<i>Escherichia coli</i>	23.0 ± 0.82	27.0 ± 0.29
<i>Salmonella typhi</i>	24.0 ± 0.00	28.0 ± 0.00
<i>Staphylococcus aureus</i>	22.0 ± 0.82	27.0 ± 0.29
<i>Pseudomonas aeruginosa</i>	21.0 ± 0.29	26.0 ± 0.00
<i>Proteus species.</i>	24.0 ± 0.82	27.0 ± 0.29

Values are Mean±S.E.M for three 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).

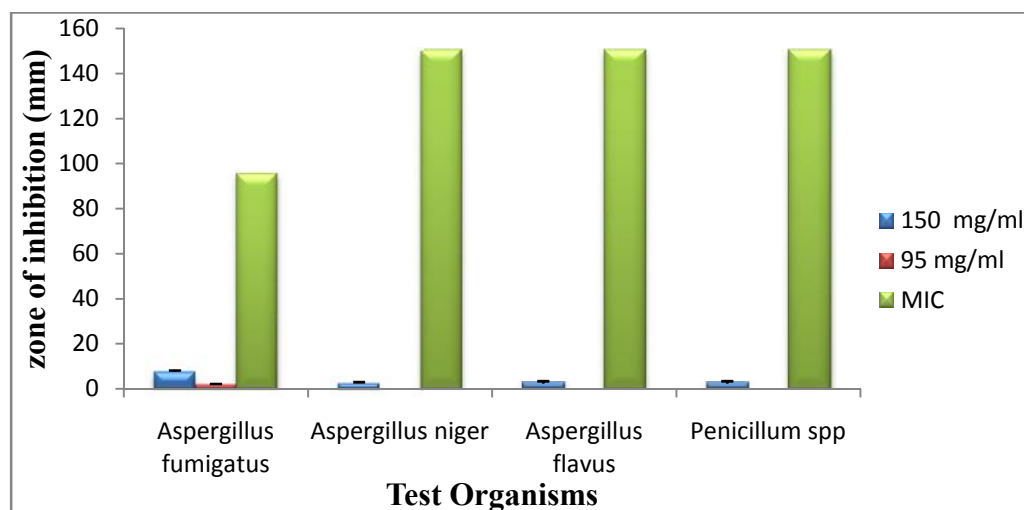


Fig 1: Minimum Inhibitory Concentration (MIC) of *Allium sativum* on fungal strains at different concentrations.

From the figure above, *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*

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139 DISCUSSION

140 The susceptibilities of the test organisms to plant extracts as reported in this study (Table 1)
 141 shows that *Aspergillus fumigatus* and *Aspergillus niger* differs significantly due to its highest
 142 zone of inhibition of methanolic extract of *Allium sativum* which is a potent antibiotic and can be
 143 particularly effective against certain fungal infections ^[12]. The minimum inhibitory concentration
 144 (MIC) of the fungal strain to plant extracts as reported in this study (Fig 1.) shows that
 145 *Aspergillus fumigatus* was inhibited at the lowest concentration (95mg/ml) of the *Allium sativum*,
 146 this can be interpreted as the extract having biostatic effect on the organism ^[13] *Aspergillus niger*
 147 was inhibited at the highest concentration (150mg/ml) of *Allium sativum*. This implies that
 148 *Allium sativum* may be very useful in treatment of Aspergillosis caused by *Aspergillus*
 149 *fumigatus*, *Aspergillus flavus* and *Aspergillus niger* ^[14] and Otomycosis (ear infection caused by
 150 *A.fumigatus*) Otomycosis can cause pain, temporary hearing loss, and in severe cases, damage to
 151 the ear canal and tympanic membrane. However, *Aspergillus flavus* was inhibited at the highest
 152 concentration (150mg/ml) of *Allium sativum* and *Penicillium spp* was inhibited at the highest
 153 concentration (150mg/ml) of *Allium sativum* (Fig 1).

154 The antibacterial activity of methanolic extracts of *Allium sativum* (Table 2) showed potent
 155 inhibition on the bacteria strains used which includes *Escherichia coli*, *Pseudomonas*
 156 *aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Proteus species* and the control
 157 (Ciprofloxacin). These findings supported the use of methanolic extracts of *Allium sativum* in
 158 herbal medicine ^[15]. Also, the antibacterial potential of *Allium sativum* supported its use as an
 159 antiseptic after birth ^[16]. It could also be seen that the methanolic extract of *Allium sativum* is

160 active against *Pseudomonas aeruginosa* thereby justifying its use for wound healing. *Escherichia*
 161 *coli* are known to cause diarrhoea and so the activity of methanolic extract of *Allium sativum*
 162 against these bacteria justifies its folkloric use in treatment of diarrhoea and urinary tract
 163 infection like gonorrhea.

164 CONCLUSION

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

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