



Research Article

Antimicrobial Activity and Phytochemical Composition of *Allium sativum* Bulb Extracts against Selected Gram- Negative Bacteria

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ABSTRACT

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As a result of increasing antibiotics resistance due to multidrug resistance leads to the use of the medicinal plants as alternative antimicrobial agents. The study aimed to investigate the antimicrobial and phytochemical properties of *Allium sativum* bulb extracts against some selected gram-negative bacteria isolates. *Allium sativum* oil was extracted using Soxhlet extraction procedures; Clinical bacterial isolates were collected from pathology department of General Hospital Potiskum in Yobe state. Isolates were identified using standard methods. Antibacterial activity of different concentrations (20, 40, 60, 80 and 100) % of oil extract of *Allium sativum* bulb were investigated against five clinical bacterial isolates including, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella boydii* and *Klebsiella oxytoca*. The oil extract of the bulbs showed antimicrobial activity against all five clinical isolates. MICs and MBCs were evaluated by broth dilution method. The minimum inhibitory concentration (MIC) values for all the three oil extracts ranged between 0.5 and 1.0%, while the minimum bacterial concentration (MBC) values ranged between 1.0 and 1.5%. Important bioactive constituents were present in the different oil extraction method; alkaloids (for ethanol extracts, 19.04±0.04, for hexane extracts, 20.04±0.14 and for chloroform extracts, 20.04±0.07) and 0.51±0.01 were detected for sterols in all the three oil extraction methods while others such as flavonoids, saponins, tannins and phenols were not detected. The yield extracts of the oil extract of the bulbs, for hexane were 12.6%, chloroform, 15.2% and ethanol, 16.2%, indicating that ethanol was the better of the three solvents used. The potential applications of *Allium sativum* oil extracts as natural antimicrobial agents call for the importance of further investigation into their nutritional potential.

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INTRODUCTION

Medicinal plants are effective source of natural antimicrobial products and their use all over the world. They are very cheap, available and renewable source of pharmacologically active substances that have long been utilized as sources of therapeutic agents worldwide due to their constituent secondary metabolites. However, plants are known to produce certain chemical that are compounds of carbon, hydrogen, oxygen and nitrogen which are naturally toxic to bacteria and other microorganisms (Singh *et al.*, 2013). "Spices are dried, fragrant, aromatic, or pungent substances derived from various plant parts, such as roots, bark, leaves, bulbs, stems, flowers, and seeds. They contribute to the flavor and seasoning of food and beverages (Mishra *et al.*, 2010).

Spices are important agricultural products which are planted globally. The spices are majorly produce from roots, bark, leaves, bulbs, stems, flowers and seeds of certain plants and mostly grown and harvested throughout the seasons of the year worldwide (Aguilera *et al.*, 2000). The plant kingdom serves as an important reservoir of biologically active compounds with various chemical structures. Many of the indigenous plants used as spices and food, knowledge of their chemical constituent becomes very important, not only for their nutritive value, but also for discovering new sources of drugs for the treatment of antibiotic resistance infectious agents. Additionally, when used spices for medicinal purpose to enhance wellbeing, their values can be observed from the phytochemical components they possess. "These phytochemicals, including steroids, flavonoids, alkaloids, tannins, and phenolic compounds, have been observed to be present in small quantities as secondary metabolites" (Okwu, 2001).

The essential oils extracted from spices and herbs have been generally recognized as containing active antimicrobial compounds such

as isothiocyanate, allicin, thymol, eugenol and carvacrol which inhibit the growth of both gram positive and gram negative bacteria (Shelef, 1983), as well as prevent fungi growth in addition to adding flavour and aroma to baked products (Azzouz and Bullerman, 1982; Paster *et al.*, 1995;). "Furthermore, Foods containing these phytochemicals not only can provide our diet with certain antioxidant vitamins but can also provide a complex mixture of other natural substances with antioxidant capacity (Sharififar *et al.*, 2009).

Allium sativum (garlic) belongs to the *Alliaceae* family and is primarily known for its bulb, which is used as a spice in various cuisines worldwide. More importantly, "Garlic has a long history of use in folk and traditional medicine, but limited documentation exists regarding its antimicrobial and phytochemical properties (Rivlin, 2016). The study aim to investigate the antimicrobial and phytochemical properties of *Allium sativum* bulb extracts, which will improve the available data on its medicinal and phytochemical value.

MATERIALS AND METHODS

Source of *Allium sativum* bulbs*Allium sativum* bulbs were source from Damaturu market. Sample of the bulbs were sent to herbarium section of the department of plant Biology, Bayero University Kano for identification with allotment of accession numbers BUKHAN 29.

Preparation of Sample

The bulbs were sun dried for seven days, after which they were pounded into powdered using mortar and then sieved through a 2.0 mm filter and subsequently stored in an air tight sterile container until it was used.

Extraction of oil from the bulbs

Allium sativum oil was extracted using Soxhlet extraction process as described by (John, 2012). Five Hundred Milliliters (500ml) of chloroform, ethanol and hexane' were poured in to a round bottom flask attached to a reflux condenser. Six pieces of anti-bumping granules were added and

50g of the prepared powdered sample were inserted in the center of the extractor. These granules are typically added to prevent vigorous boiling and bumping of the solvent during the extraction process. The extraction was carried out at 61.2^oC; 78.4^oC and 69.0^oC for chloroform, ethanol and hexane respectively, these temperatures were selected based on the boiling points of the solvents to optimize extraction efficiency. When the solvent boil, the vapor rose through the vertical tube in to the condenser at the top. The condensed solvent flows down the condenser and drips onto the solid sample in the thimble, allowing for continuous extraction. The extracts sip through pores of the thimble and fill the "collection flask", where it flows back down in to the round bottom flask quick fit. The extraction was carried out until the solvent in the round bottom flask became clear or colorless. It was removed from the tube, dry in the oven, cool in the desiccators and then weigh again. These steps are typically performed to remove any remaining solvent and ensure accurate determination of the extract's weight. Additional extractions were performed as needed. At the end of extraction process the resulting filtrate was left to evaporate at room temperature to remove the solvent and obtain the final extract.

Samples of Bacteria

Clinical bacterial isolates from samples of urine, blood, sputum, catheter tips, stool, urogenital, and abscesses were collected from pathology department of General Hospital Potiskum in Yobe state. Isolates were identified using several biochemical procedures as described by Cheesbrough (2017). The species of bacteria identified were, *E. coli*, *Shigella boydii*, *Klebsiella oxytoca*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The cultures of bacteria were maintained on nutrient agar slants at 4^oC.

Phytochemical screening of oil extract

The oil extracts obtained from the bulbs were subjected to phytochemical screening to determine the presence of alkaloids, flavonoids, phenolic compounds, saponins, steroids, and tannins. The screening was performed following the methods previously described and highlighted by Enabulele and Ehiagbonare (2011).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

The MIC was determined using the tube dilution method. Standardized suspensions of the test bacteria were inoculated into a series of sterile tubes of nutrient broth containing different concentrations of oil extracts and incubated at 37^oC for 24hours. The MICs were read as the least concentration that inhibited the growth of the test bacteria (Kamba, and Hassan, 2010). The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was sub-cultured onto oil extract free agar plates, incubated for another 24 hours at 37^oC. The minimum bacterial concentration (MBC) was considered as the lowest concentration that will not produce a single bacterial colony (Kamba, and Hassan, 2010).

RESULTS

Results of the study were presented in tables 1 to 4. The results showed the presence of important Phytochemical and significant antimicrobial activity against the bacteria tested. The percentage yield of the oil extract of the *Allium sativum* bulbs were, 12.6% for hexane, 15.2% for chloroform and 16.2% for ethanol indicating that ethanol was the better of the three solvents used (table 1).

Table 1: Percentage yield of the oil extracts of *Allium sativum* bulbs

Extraction Solvent	Bulb powder(g)	Weight of extract(g)	Yield (%)	Colour	Texture
Hexane	50	6.3	12.6	Light yellow	Oily
Chloroform	50	7.6	15.2	Light yellow	Oily
Ethanol	50	8.1	16.2	Light yellow	Oily

Results of Phytochemical screening of the oil extracts

Allium sativum bulbs revealed the presence of some important bioactive components as presented in table two (Table 2). It contains very

high amounts of alkaloids in all the solvents extraction methods (ethanol, hexane and chloroform), while sterols were present in trace amounts. Saponins, tannis, phenols and flavonoids were not detected in all the extracts.

Table 2: Phytochemical Constituents of oil extracts of *Allium sativum* bulbs

Constituents	Solvents (mg/ml)		
	Ethanol	Hexane	Chloroform
Alkaloids	19.04±0.04	20.04±0.14	20.04±0.07
Sterols	0.51±0.01	0.51±0.01	0.51±0.01
Tannis	Not detected	Not detected	Not detected
Saponins	Not detected	Not detected	Not detected
Flavonoids	Not detected	Not detected	Not detected
Phenols	Not detected	Not detected	Not detected

Results of the antibacterial activity of the oil extracts

Allium sativum oil extracts against clinical isolates namely *E. coli*, *Shigella boydii*, *Klebsiella oxytoca*, *Proteus vulgaris* and *Pseudomonas aeruginosa* at different concentration are presented in Table 3.

Table 3: Mean zones of inhibition of *Allium sativum*(bulb) oil against bacteria

Bacteria	<u>Zones of Inhibition in mm at diff different concentrations (percentage of control) with standard error of mean</u>															
	20% Hexane	20% Chloroform	20% Ethanol	40% Hexane	40% Chloroform	40% Ethanol	60% Hexane	60% Chloroform	60% Ethanol	80% Hexane	80% Chloroform	80% Ethanol	100% Hexane	100% Chloroform	100% Ethanol	Control Amikacine 30mg
<i>E. coli</i>	11.00 ±0.00	11.27 ±0.60	11.33 ±1.16	18.33 ^c ±1.56	19.63 ±1.06	19.33 ^c ±1.16	22.00 ±0.06	22.20 ±1.06	22.00 ±0.00	22.00 ±0.58	22.72 ±1.52	22.00 ±0.00	25.60 ±0.89	25.82 ±0.19	25.00 ±0.00	12.05 ±4.73
<i>P. vulgaris</i>	2.67 ±2.89	2.87 ±2.59	1.67 ±2.89	2.89 ±0.62	2.89 ±6.87	2.67 ±4.62	4.10 ±6.03	4.32 ±0.03	4.00 ±6.93	5.08 ±6.07	6.08 ±0.00	5.00 ±8.67	5.97 ±5.82	6.77 ±4.82	5.67± 9.82	12.33 ±1.06
<i>P. aeruginosa</i>	7.33 ±4.78	8.53 ±4.68	7.33 ±6.43	10.00 ±9.00	11.00 ±6.40	10.00 ±8.66	19.00 ±5.03	20.00 ±0.03	18.67 ±5.77	23.33 ±4.69	23.65 ±4.27	23.33 ±2.87	26.40 ±1.53	26.60 ±5.93	26.00 ±1.73	15.33 ±1.45
<i>S. boydii</i>	0.00 ±0.00	1.00 ±0.00	0.00 ±0.00	2.00 ±0.00	2.60 ±0.00	1.67 ±2.87	4.33 ±6.28	5.36 ±6.20	4.33 ±4.04	10.00 ±9.00	11.00 ±0.00	10.00 ±0.00	12.85 ±3.16	12.95 ±6.16	12.67 ±1.16	11.33 ±1.00
<i>K. oxytoca</i>	00.00 ±0.00	1.20 ±0.00	0.00 ±0.00	6.33 ±8.03	7.53 ±2.03	6.33 ±6.03	11.83 ±6.21	12.03 ±6.21	11.67 ±6.51	16.52 ±6.11	17.52 ±3.41	16.33 ±6.11	20.89 ±3.00	21.69 ±2.00	20.67 ±4.62	13.33 ±1.32
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Results of MIC and MBC

The minimum inhibitory concentration (MIC) values for all the three oil extracts ranged between 0.5 and 1.0%, while the minimum bacterial concentration (MBC) values for all the

three oil extraction ranged between 1.0 and 1.5%. MIC and MBC are values normally used as predictive indices of the efficacy of antimicrobial agents as presented in Table 4.

Table 4 MIC/ MBC activities of *Allium sativum* (Gallic) extract against bacteria isolates.

Organisms	Ethanol Extraction		Hexane Extraction		Chloroform Extraction	
	MIC%	MBC%	MIC%	MBC%	MIC%	MBC%
<i>Escherichia. Coli</i>	0.5	X	1.0	1.5	0.5	1.0
<i>Proteus vulgaris</i>	0.5	1.0	0.5	1.5	0.5	1.0
<i>Pseudomonas aeruginosa</i>	1.0	1.5	1.0	1.5	1.0	1.5
<i>Shigella boydii</i>	0.5	X	0.5	1.5	0.5	X
<i>Klebsiella oxytoca</i>	0.5	1.5	1.0	1.5	0.5	1.0

KEY;

MIC: minimum inhibitory concentration. MBC: minimum bactericidal concentration.

X: MBC above 1.5%

DISCUSSION

Results obtained from the percentage yield of the oil extract of the *Allium sativum* bulbs indicating that ethanol was the better of the three solvents used (table 1). This observation may be attributed to the factors such as age, type of plant material and polarity of the solvent used, may affect extract yield as reported by (El-Mahmood *et al.*, 2009).

Phytochemical screening of the oil extracts of *Allium sativum*

Bioactive compounds detected (alkaloids and sterols) in this study have been severally proven to be active against human pathogens (El-Mahmood, 2009; Enabulele, and Ehiagbonare, 2011). Apart from their potential antimicrobial activity, bioactive phytochemical compounds found in this study such as alkaloids are known as ant-malaria agents, analgesics and can act as stimulants. Generally the effectiveness of any antimicrobial compound depends on the ability of the antimicrobial to inhibit the growth of any

bacteria in the body system they infect. Because of the high genetic differences of bacteria, they seem to however develop the ability to rapidly evade the action of antimicrobials by becoming resistant to them. It becomes necessary therefore to consistently look for alternative methods of eliminating microbial threat that causing infections.

Minimum inhibitory concentration and Minimum bactericidal concentration

The differences in the effectiveness of the oil extracts as presented in Table 4 could be due to the crude nature of plant extracts which may contain some impure substances that may be inert and do not have antibacterial activity. Unlike the synthetic antibiotics whose active ingredients are in their pure state (El-Mahmood, 2008).

CONCLUSION

The study revealed that *Allium sativum*, oil extracts contain important compounds such as allacin, ajoene, and diallyl sulfides, which are known for their antibacterial properties. For

example oil extracts were active against clinical bacterial tested (*E. coli*, *Shigella boydii*, *Klebsiella oxytoca*, *Proteus vulgaris* and *Pseudomonas aeruginosa*). The phytochemicals found in *Allium sativum* oil extracts, such as alkaloids and sterols have potential health benefits and contribute to the overall medicinal

value of the plant. Further studies need to be carried out on the nutritional benefit of the oil extracts such as vitamins, minerals, or antioxidant content so as to maximize the medicinal and nutritional potential of the plant bulb for dietary supplements, or pharmaceutical formulations based on the oil extracts.

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