Original Research Article

HERBOMINIRALS AND ANTIBACTERIAL ACTIVITIES OF *Allium sativum* L EXTRACTS ON PATHOGENIC BACTERIA CAUSING MENINGITIS IN SUB-SAHARAN AFRICA, ZARIA, KADUNA STATE, NIGERIA

ABSTRACT

Bacterial Meningitis (BM) is the most common serious infection of the central nervous system (brain and spinal cord). This research aims to determined herbominirals and to evaluate the in vitro antibacterial activity of the extracts (JEAS, EEAS and AEAS). The collected bulbs of A. sativum (600 g) were washed and air dried under shade for 2 hours and the dry scaly outer covering was peeled-off to obtain the fresh garlic cloves which were then divided into three parts of 200 g each. These three portions were crushed separately for cold extraction. The first portion was homogenized and poured into a muslin cloth to squeeze out the juice, while second and third portions were homogenized and submerged into 500 ml of 96% ethanol and 500 ml of distilled water respectively for 24 hours and both filtered after thorough shaking. The antibacterial activity of bulbs of A. sativum juice, ethanolic and aqueous (JEAS, EEAS and AEAS) extracts as folkloric medicine against Neisseria meningitides, Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae and Escherichia coli were determined using Agar well diffusion and broth dilution method. Distilled water, concentrated nitric acid (HNO3) and hydrochloric acid (HCl) were used to digest the extract, which was then heated in water bath at 90oC and filtered to obtain the filtrate for the analytical studies for A. sativum nutritional composition and zeolite herbominerals. The micro-herbominerals with their proximate values were observed were Nano pharmacologic of Silver, Manganese, Zinc, Iron and Selenium; which exerts biocidal properties as well as stimulates immune system to cushioning the challenges of the BM pathogens. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and phytochemical screening of the extracts were evaluated. The results obtained showed that the juice and ethanolic extracts were potent, inhibiting the growth of clinical isolates with zone of inhibition ranging from 14-36mm.The extracts inhibited bacterial isolates in the concentration dependant manner with MICs ranging between 0.02-15mg/ml and MBCs between 0.04-5mg/ml respectively. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, anthraquinone, carbohydrates, fats and oils, steroidal ring, saponins and terpenoids. This experimental investigation has provided the scientific validation basis for the ethnomedical use of A. sativum as a remedy to treat bacterial meningitis locally as anti-infectious chemotherapeutic locally.

Keywords: Herbominirals, Antibacterial, *Allium sativum*, Bacterial Meningitis, Sub-Saharan Africa, Kaduna State.

1.0 INTRODUCTION

Bacterial Meningitis (BM) is the most common serious infection of the central nervous system (pia mater and arachoid inflammation called leptomeningitis and inflammation of brain parenchyma called encephalitis) and spinal cord (myelitis) [1]. Bacterial endotoxins in the blood infect the pia mater, arachnoid and subrarachnoid space leads to cerebrospinal fluid (CSF) meningeal irritation and the clinical trial of headache, fever and meningism [2]. Antibiotics and vaccines meant to reduce morbidity and mortality of BM as modern therapy are available in BM radical treatment. Despite all these efforts put in place, BM remains associated with an unacceptable rate of high morbidity and mortality in Sub-Saharan Africa, including Nigerian Northern States [3].

Aside of the massive side effects associated with the conventional antibiotics; there has also been the emergence of resistant strains of the infecting organisms probably from the frequent irrational use of the available antibiotics to treat conditions of unknown aetiology. And often resistance hampers the control of bacteria in general and tends to increase cost of healthcare services for bacteria chemotherapeutics [4].

Therefore, bacterial meningitis (BM) is a devastating air-borne infectious disease of humans, and it is of huge health burden as it can easily kill or maim when meningeal pathogens invaded blood stream. The BM causes cellular hemorrhagic sepsis, metabolic, endocrine and neurologic disorders. This burden of the disease is highest in the developing countries and especially in the immunity compromised rural populations [5]. Inflammation of the meninges causes leakage of the infected

cerebrospinal fluid (CSF) and alteration of the brain system (cognitive deficit) giving rises to meningism (stiff neck, severe headache, fever, rashes, shortness of breath or noisy breathing, hydrocephalus (swelling or oedema of the brain microglia and astrocytes due to inappropriate antidiuretic hormone secretion in the brain parenchyma and leptomeninges).

High cost of acquiring synthetic antibiotics and the inconveniences of seeking for effective treatment due to using exorbitantly expensive drugs make life difficult for people, considering the economic status of an average Nigerian as a low income earner [3]. Moreover re-emergence of BM persists in epidemic meningitis belt of Africa. Therefore, to overcome BM, new knowledge of natural products against BM pathogens is urgently needed; especially new drugs are required for BM mitigation which differs from existing medicines [4].

As for the prevalence of meningeal bacterial organisms, the potential of epidemic virulence of meningeal bacteria organism in Nigeria varies with the geographical location, time of season, climate and pathogenic serotype strains of the causative organism [3]. Six subspecies serotypes of *meningococci* (A, B, C, W-135, X and Y) have been clinically recognized in Sub-Saharan African countries of which serotypes A and C *meningococci* were found to be more prevalent and predominant among other serotypes which often occur as co-morbid infections [1]. The epidemics of Sudan in 2006 was of serotypes W-135 and X [1], 2009). The most prevalent meningeal bacteria organism in Nigeria is of the serotype A and C strains of *Neisseria meningitides* seen abundantly during the harmattan period when humidity is very low, usually around November-June and which is the breeding time of the organisms. *Neisseria meningitides* is the most implicated bacterial organism that causes meningitis and constitutes about 80% of the epidemic cases in Africa [6]. Other bacterial organisms (*Haemophilus influenzae* type b, *Streptococcus*

pneumoniae, Escherichia coli, Klebsiella pneumoniae) also cause meningitis to a lesser extent (about 20% of the epidemic cases) [6, 7]. Thus, these five bacterial organisms are those mostly known to be involved in meningitis, systemic septic-inflammations and hemorrhage in Sub-Saharan Africa including Nigeria [6]. Bacterial meningitis caused by *H. influenzae* and *meningococci* have a better prognosis than of *S. pneumoniae* and *E.coli* that mainly attack neonates and growing children. The group B *streptococci* (subtype III) occur mainly during the first week of life (newborns), while *Escherichia coli* and *Klebsiella pneumoniae* are of both newborns and growing children less than five years of age. *Haemophilus influenzae* type B, *Neisseria meningitides* (meningococci) and *Streptococcus pneumoniae* affect mostly the adults, but people over 50 years have increased risk of *Escherichia coli* [3].

1.1 African BM Epidemiology

Annual epidemic of BM occurs during the hot dry season in Sub-Saharan African countries while BM strikes during the cold rainy season in Western countries [8]. About 1.2 million cases of BM annually worldwide with 135,000 deaths and is now a 'top10' infectious disease cause death worldwide and half survivors deformed [5]. African BM (epidemic) ranges from 100 to 1000 per 100,000 populations and over 3,000 populations per day were infected during explosive meningitis belt while 20 per 100,000 populations in the western world (USA, UK and Europe) [7]. Thus, African BM (non-epidemic) ranges from 10 to 20 per 100,000 for Sub-Saharan African countries **while** 1 to 5 per 100,000 for western countries [9].

1.2 The Pathogenesis and Pathophysiology of Bacterial Meningitis

BM involves a complex interplay between virulent factors of the pathogens and the host immune responses. Therefore, BM develops when virulent factors of the

pathogen overcome host defense mechanisms. Meningeal bacteria usually weaken the functions of the immune defense system which predisposes the body system to damaging opportunistic infections. Bacterial infection in particular often leads to septicaemia or persistent multiplication that traumatizes the blood vessels. Bacteria lyses, immune activator binding, leucocyte infiltration causes glial cells and astrocytes to be inflamed, inflammatory factors as TNF, COX-2, NK, IL, iNO, and Phospholipase A2 which triggered meningeal inflammation. Neuropathologic effect of BM, brain oedema formation due to brain parenchyma inflammation, impairment of CSF hydrodynamics (inappropriate secretion of antidiuretic hormone, IASADH), increased intracranial pressure leads to hydrocephalic head in neonates [10, 3]. BM is pathologically characterised by metabolic, endocrine, neurologic disorders and amnesia as well as dysfunctions of multiple organs such as heart, liver, kidneys, and lungs [1].

In the extreme conditions involving intravascular coagulations, organ failure and / or sudden death may occur related to the rapid shutdown of the vital organs. Haemorrhage is a severe form of blood loss that can cause general shock as a result of reduced tissue perfusion and / or ischaemia [11, 10]. Meningeal bacteria causing reduction in the levels of blood clotting cells (platelets), this leads to severe erythematous purpura, DIC and uncontrollable bleeding which can be internal haemorrhage in the vital organs of the body [12]. These blood clots can reduce or block blood flow through the body blood vessels due to bacteria coagulases lead to the damage and sudden death of body's organs. In DIC, the increased clotting uses up platelets and clotting factors in the blood, it deregulates normal physiologic mechanisms of the blood. Furthermore, it can cause internal and external bleeding in meningitis patients mainly infants [10]. Thus, internal bleeding occurs inside the body while external bleeding occurs underneath or from the skin or mucosa. These

meningitis pathogens cause sepsis of the blood, fever, diarrhoea and sudden death [10].

Pains (abdominal, chest), swelling, hemoptysis (bloody vomitus), rashes, photophobia (light intolerance),hyperthermia, hypoglycemia, convulsion/seizures, phonophobia (high noise intolerance), drowsiness, confusion, clouded vision, loss of consciousness, arthalgia, altered mentation, sight and hearing loss and crippled limbs [12, 6].

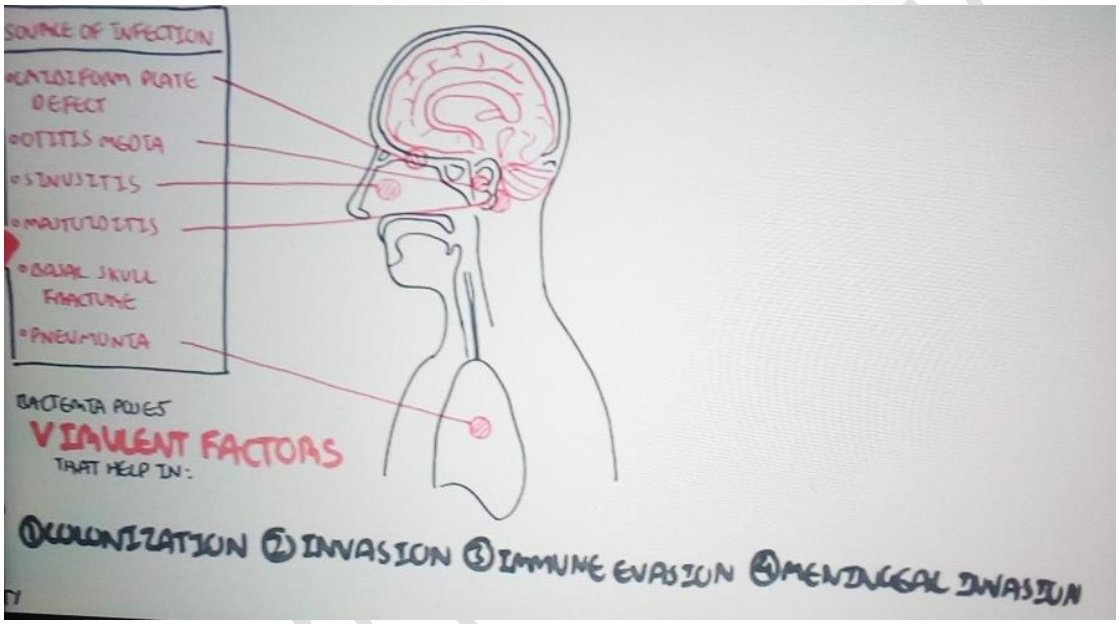

Figure 1: shows the pathogenesis of bacterial meningitis.

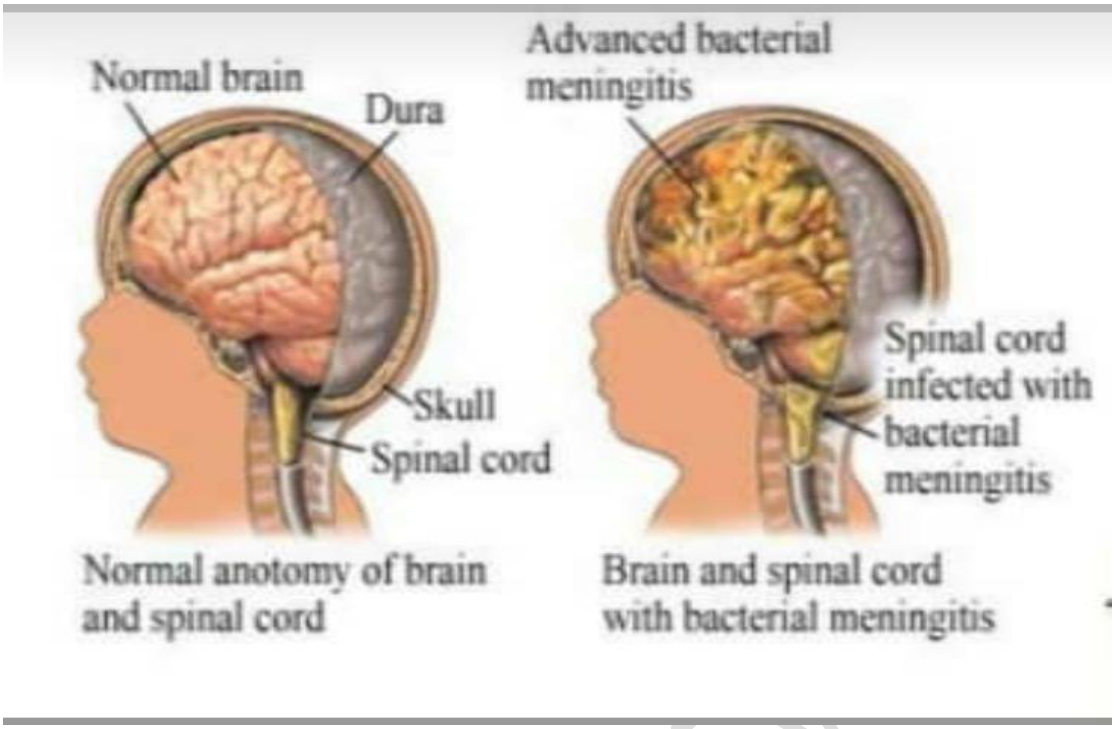

Figure 2: shows the anatomical pathogenesis of bacterial meningitis. Adapted from www.anatomyatlas.com

1.3 Aetiologic Factors of Bacterial Meningitis

- Malnutrition
- Climate change usually hot weather
- Overcrowding (as in crowded quarters, or gatherings with infected persons who most times are asymptomatic carriers of the pathogens)
- Deficient immune system
- Poor sanitation (environmental hygiene) that breeds the causative organisms
- Symptoms of inappropriate antidiuretic hormone secretion (SIADHS) in bacterial endotoxin-induced oedema of the body due to endocrine crises [5, 3].

However, *A. sativum* (AS) has a long history of use as food condiment and also a high value of use traditionally for many ailments including antimicrobial effects. However, its specific activity on meningeal bacteria as well as antihaemorrhagic activity in condition of meningococcal-induced cellular injuries has actually not been reported in

literature. Also, a scientific validation of this utilization has not been previously made to the best of my knowledge [3].

1.4 Description and Origin of *Allium sativum*

African Traditional medicine constitutes an important source of drugs for ethnopharmacological investigation. Thus, Traditional medicine by WHO definition is the sum total of the knowledge, skills, and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in maintenance of health in prevention, diagnosis, improvement or treatment of physical and mental illnesses [13]. Various medicinal food-plants and animal products-supplements are available for use in certain immune-deficiency disease conditions related to malnutrition such as hemorrhagic sepsis. Furthermore, phytotherapy still remains a habitual part of health care system wholly or in part especially in rural communities [3]. *Allium sativum* (AS) is one of such medicinal food-plant that has a long history of use as food condiment and it also has a high value of use traditionally for many ailments including typhoid fever, ulcer, cholera, dysentery etc and thus, there is a need to investigate its specific *in vitro* antibacterial activity on meningeal bacterial organisms as well as in condition of meningococcal-induced cellular injuries having been known to boost the immune system [14].

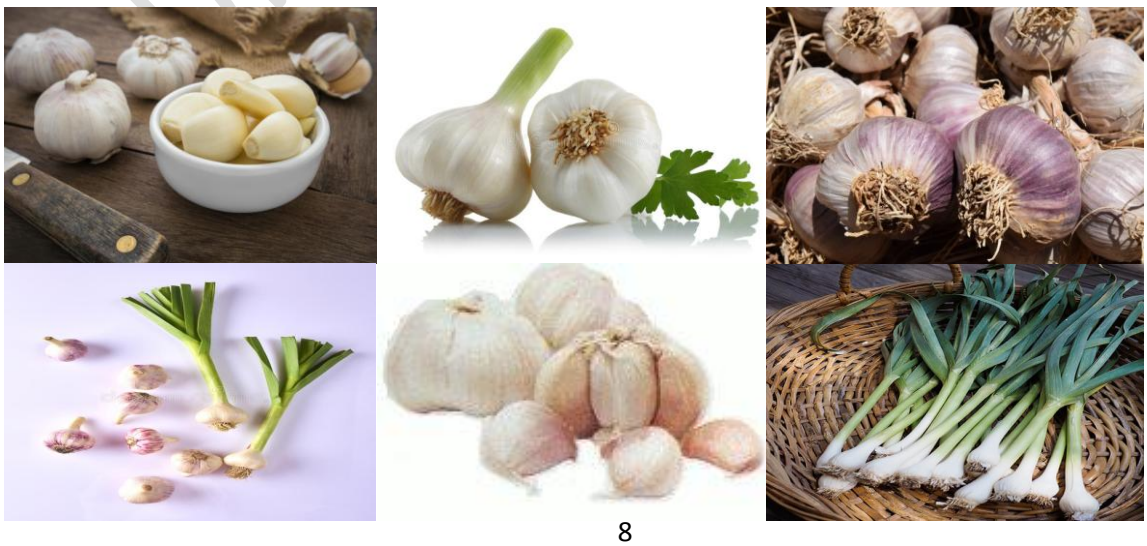

Figure 3: Garlic Plant in its Natural Habitat, Source: Rader and McGuinness, [15]

Scientific name: *Allium sativum* Linn

Family: Lilliaceae

Common name: Garlic

Hausa name: *Tafarnuwa*

Yoruba name: *Allubosa ayu*

Igbo name: *Allibasa ayo*

Ebira name: *Allivasa ahono* [3].

1.5 Folkloric and Ethnomedical Uses

A. sativum has an ancient use as antiseptic, antibacterial, carminative, bleeding of the nose, diaphoretic, anthelmintic, antimicrobial, antiflatulence, and analgesic. It is also used locally for confluent smallpox, typhoid fever, dropsy or congestive heart failure, pulmonary tuberculosis or phthisis and for eye and ear infections [16]. It has also been reported to have antitumour and anticholesterol-anemia properties whereby it is used to decrease low density lipoprotein and / or increase high density lipoprotein as to lower blood pressure. In China and Arabia, it has a history of use as energizer or palliative and it is believed to have a natural nutritional healing power for cough, hay fever, pile or dysentery and also to promote digestion (dyspepsia) [16,17].

2.0 METHODOLOGY

2.1 Collection and Identification of Plant Material

The bulbs of *A. sativum* were obtained as fresh farm products from Valiki Market, Okene, Kogi State, Nigeria in November, 2014. The plant's identification was authenticated by Mallam Umar S. Gallah at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and compared with voucher specimen number of 2156 for future reference.

2.2 Extraction of Plant Material

The collected bulbs of *A. sativum* (600 g) were washed and air dried under shade for 2 hours and the dry scaly outer covering was peeled-off to obtain the fresh garlic cloves which were then divided into three parts of 200 g each. These three portions were crushed separately for cold extraction according to the method of Fattouch *et al*., [18]. The first portion was homogenized and poured into a muslin cloth to squeeze out the juice, while second and third portions were homogenized and submerged into 500 ml of 96% ethanol and 500 ml of distilled water respectively for 24 hours and both filtered after thorough shaking. The first and second portions were freeze dried, while the third portion was evaporated over water bath at 50^{o}C to obtain the powdered yield. The three samples obtained were then stored in separately labeled air-tight container for later use. The percentage (%) extract yield was calculated as follows:

% yield = weight of extract (g) / weight of garlic cloves (200 g) x 100

2.3 Preparation of the Extracts

Working concentrations to be used for the study were prepared from the obtained extracts (JEAS, EEAS and AEAS) using distilled water as a diluent. The required

various concentrations to be used for each experiment were always freshly prepared from the stock solution of the extracts.

2.4 Test Clinical Bacteria Isolates

Five pathogenic clinical isolates (three anaerobic organisms): *Neisseria meningitides, Streptococus pneumoniae, Haemophilus influenzae* and (two aerobic organisms) *Klebsiella pneumoniae* and *Escherichia coli* obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria in Kaduna state, Nigeria were cultured in Mueller Hinton Agar (MHA) to obtain colonies of the organisms as described by Monica [19] and Sharma and Aneja [20].

2.5 Phytochemical Screening of *Allium sativum*

The methods of [13] were used to screen for the presence of various phytochemical constituents in the *A. sativum* extracts. Phytochemical constituents screened include alkaloids, anthraquinones, cardiac glycosides, carbohydrates, fat and oils, flavonoids, saponins, steroids, terpenoids and tannins. 2.0g of powders of each of the three extracts were dissolved in 100ml of distilled water for this screening experiment.

2.6Herbominiral Analysis and Proximate Values of *Allium sativum* Extract (JEAS)

Analytical automated instruments including spectronic 21D / flame photometer, unicam 969 AA spectrometer (AAS), fast sequential atomic spectrometer AA240FS and AA spectrophotometer Buck205 were used to analyze their proximate values in the JEAS according to the methods described by Naturopathy [21]. Distilled water, concentrated nitric acid (HNO3) and hydrochloric acid (HCl) were used to digest the extract, which was then heated in water bath at 90^{o}C and filtered to obtain the filtrate for the analytical studies for *A. sativum* zeolite herbominerals. The presence of dietary

phytonutrients including carbohydrates, proteins, dietary fibres, fats and oils, vitamins as well as the zeolite herbomineral elements and their proximate values were determined. 2.0g of JEAS powder was dissolved in 100ml of DH2O for the quantitative determination of vitamins, macro and micro-elements and other nutrients. 10ml for vitamin analysis (200mg), 30ml for macro/micro (zeolite) element analysis (600mg) and 30ml for other nutrients analysis (600mg) respectively.

2.7 Determination of Antibacterial Activity of *Allium sativum* Extracts

The bacterial cultures were prepared by transferring with sterile wire loop, each bacterium cell into a Nutrient Broth Medium which was incubated at 37^{o}C for 24 hours as described by WHO-BS [22] to obtain a colony of the bacterial cells. The cells were maintained in Nutrient Broth Media in universal bottles labeled for each bacterium. Three tubes (1, 2, 3) each containing 9 ml of normal saline were set up for 3-fold serial dilution (1:1000) for the gram-positive bacterium, in which 1 ml of the overnight culture was transferred into the first test tube and mixed; and 1ml of this then taken into the second test tube from which another 1ml was removed into the third tube which was then deemed to be 1x10^{3} colony forming unit / ml (CFU/ml). The same serial dilution, but in a fourth tube containing 4.5 ml of normal saline and 4-fold dilution (1:5000) was also performed for the gram-negative bacteria to the last concentration of 1x10^{5}CFU/ml. The last diluted concentrations (5 test tubes of the bacterial organisms) were incubated at 37^{o}C for 24 hours after which, the bacterial organisms (suspension) were inoculated on prepared Molten sterile Mueller-Hinton agar plates by taking 2 ml of each of the organisms and flooding it over the agar surface by agar well diffusion method of Sharma and Aneja, [20]. Four different concentrations (10, 15, 20 and 25 mg/ml) and 5 mg/ml of the standard drug (cefuroxime axitel) were used to study the growth inhibitory effect for each of the

three extracts of *A. sativum*. Wells were bored on the agar dishes and a drop of the molten agar was used to seal the bottoms of the bored wells prior to filling them with 0.2 ml of each the various drug concentrations. The plates were then kept for 1 hour to diffuse and then incubated at 37°C for 24 hours. The experiment was performed in duplicate and the zones of growth inhibition around the two wells for each drug concentrations were measured in millimeter using a ruler. The mean of the duplicate experiments for each concentration of the extracts were then calculated and recorded as the growth inhibitory zone of the extract concentrations for each of the organisms [22, 19].

2.8 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The same extracts concentrations of 10, 15, 20 and 25 mg/ml of the three *A. sativum* were used for MIC and MBC experiments. Serial dilutions were made for each concentration as well as for the standard drug whereby ten test tubes with each containing 9 ml distilled water were set up for each concentration and 1 ml of each concentration taken into the first test tube and from which 1 ml was taken into the second test-tube and continuously like that until the last test tube after which the 1ml taken from it was discarded. Then 2 ml of each of these mixtures (varied concentrations) in test tubes were taken respectively into various petri-dishes containing 8 ml of molten agar that had been labeled for each diluted mixture and then mixed to form broth dilution. Similar dilution was carried out for each of the three extracts and in all; a hundred and fifty mixture petri-dishes were gotten (50 for JEAS, 50 for EEAS and 50 for AEAS). All of these were kept for 2 hours to solidify after which a sterile forcep was used to place 5 sterile filter paper discs of 6 mm in diameter on each solidified agar plate surface. A micropipette was used to inoculate

0.02 ml of each of the five bacterial isolates on each of the discs and this was done for all the four concentrations of the three extracts under study and also for the standard drug. The plates were then incubated at 37^0C for 24hrs. The lowest diluted concentration of each extract stock concentration in which there was no visible growth of any of the bacterial organisms was considered as the Minimum Inhibitory Concentration (MIC) for the organism in question [19]. The Minimum Bactericidal Concentration (MBC) of the various extracts were performed from the lowest concentration (MIC) of the dilutions of the 4 stock concentrations and the higher concentrations above it. For instance, the 10 mg/ml has its diluted concentration of 2.5 mg/ml as its MIC and thus, three test tubes labeled as 2.5, 5.0 and 10 mg/ml and containing 2 ml nutrient broth and 3 drops of 3% v/v TW 80 were set for MBC. Then, filter paper discs of the bacterial organisms from the corresponding MIC experiment were transferred into the test tubes respectively and again incubated at 37^0C for 24 hours and checked for growth inhibition [19, 20].

2.9 Statistical Analysis

Results obtained were recorded as mean ± SEM and subjected to one way analysis of variance (ANOVA) and where significant differences exist, means were compared using Waller Duncan test was performed using Statistical Analysis System (SAS, software version 2002) at 0.05 significant level ($P<0.05$).

3.0 RESULTS AND DISCUSSION

Results of percentage yielded of the three extracts were shown in Table 1. The results showed Ethanolic extract of *A. sativum* yielded highest percentage with light yellow powder, Aqueous extract of *A. sativum* was second yielded percentage with brown powder and Juice extract of *A. sativum* was least percentage yielded as depicted below. The results of phytochemical screening of the bulbs extracts of *A. sativum* are shown in Table 2. The results showed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, fats and oils, flavonoids, saponins, steroidal ring, terpenoides and tannins. The presence of phytochemicals in the bulbs extracts (Table 2) showed that the extracts possess strong antibacterial properties. These results are agreement with similar study by Abdullahi *et al*., [13].

Table 3, were demonstrated to possess health benefits and are used as combined tools for treating bacterial meningitis. Its zeolite herbominerals such as silver ions, zinc ions, selenium ions have bactericidal effect on bacterial meningitis pathogens through their nanopharmacologic and chelation therapy [23]. It protects entire body by stimulating the immune system, prevents infectious diseases, improves health, and supports the structures (collagens) [17].

The Table 4: shows the results of antibacterial effectiveness of A. sativum bulbs extracts against the test clinical bacteria isolates. Results showed that the activity of the bulbs extracts against the test bacteria decrease with decrease in the concentration ie antibacterial effect of the extracts are concentration dependent manner; with the juice extract demonstrating higher antibacterial activity (12.0-36.0 mg/ml) than the ethanolic extract. While aqueous extract lost activity due to heat applied because *A. sativum* active compounds are heat labile biodegradable. It has been observed that the more polar the solvent, the higher the yield of extract shown in the Table 1, but the

juice and ethanolic extracts are potent due to their freeze drying process with high bioactive principles.

Figure 4 and Table 5,6,7,8,9 showed the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the three extracts against the test clinical isolates respectively. These results showed the values obtained from experiment are quite higher for the juice extract than that of ethanolic extract, suggesting that extraction with ethanol could produce better active antibacterial phytochemicals with active organosulphorus volatile oil compounds which are contained in bulbs through freeze drying procedures. These results of the present study are in agreement with similar study by Sen *et al*., [24]; Abdullahi and Ajayi, [25]. The observed antibacterial effect of the bulbs extracts on the clinical bacterial isolates though *in-vitro* demonstration of antibacterial experiment is an indication; that *A. sativum* bulbs extracts could be effective in the management of bioterrorising infections such as BM cause by these bacteria (Table 6 and 7).

3.1 Extraction of the Plant Material

The calculated percentage yield from the 200 g of *Allium sativum* L bulbs used for the extraction was as given in table 1 below:

Table 1: Percentage Yield of *Allium sativum* Extracts

Extract	% Yield (w/w)	Observed Colouration
JEAS	12.7	light yellow powder
EEAS	17.2	light yellow powder
AEAS	14.7	Brown powder

3.2 Phytochemical Screening of the Extract

The table below is a summary of the phytochemical components or secondary metabolites of the extract. From the obtained result, anthraquinone and tannins were not present in the extract of the bulbs.

Table 2: The Phytochemical Constituents of *Allium sativum* (AS) Bulb Extracts (JEAS, EEAS and AEAS)

Phytochemical Components	Inference		
	JEAS	EEAS	AEAS
Alkaloids	Present	Present	Present
Anthraquinone	Absent	Absent	Absent
Carbohydrates	Present	Present	Present
Cardiac glycosides	Present	Present	Present
Fats and Oils	Present	Present	Present
Flavonoids	Present	Present	Present
Saponins	Present	Present	Present
Steroidal ring	Present	Present	Present
Terpenoids	Present	Present	Present
Tannins	Absent	Absent	Absent

Note: JEAS= Juice Extract, EEAS= Ethanolic Extract and AEAS=Aqueous Extract

Table 3: The Herbominiral Elements and Proximate Values of Juice Extract of Allium sativum (JEAS)

Herbominiral Elements	
Macro/Micro (Zeolite) Elements	**Proximate Values (mg)/600mg**
Sodium	19.00
Potassium	154.00
Calcium	184.00
Magnesium	26.00
Phosphorus	390.00
Sulphur	450.00
Manganese	0.03
Silver	0.01
Iodine	0.05
Zinc	1.18
Selenium	0.014

3.3 Inhibitory Effect of *Allium sativum* Extracts on Growth of Clinical Bacterial Isolates

The result obtained showed that *A. sativum* extract inhibited the growth of clinical bacterial isolates at 30mg/ml showed significant inhibitory $P<0.05$ in in-*vitro* susceptibility test compared with that of standard Drug (control) (See Table 5).

Table 4: Inhibitory Effect of *A. sativum* Extracts on the Growth of Clinical Bacterial Isolates

Test Exts.	Zone of growth inhibition (mm) of the extracts at drug concentrations (mg/ml)					Bacterial orgs
	10	15	20	25	5 (cefuroxime)	
JEAS	20.5±0.50	25.5±0.50	29.5±0.50	30.5±0.50[a]	35	*Streptococcus pneumoniae*
EEAS	-	-	-	12.5±0.50	35	
AEAS	-	-	-	-	35	
JEAS	28.0±0.00	33.5±0.50[a]	34.0±0.00[a]	36.0±0.50[a]	35	*Neisseria meningitides*
EEAS	-	-	14.5±0.50	17.5±0.50	35	
AEAS	-	-	-	-	35	
JEAS	-	-	-	12.5±0.50	20	*Klebsiella pneumoniae*
EEAS	-	-	-	-	20	
AEAS	-	-	-	-	20	
JEAS	23.0±0.00	26.5±0.50	28.0±0.00	30.5±0.50[a]	35	*Haemophilus influenza*
EEAS	-	15.5±0.50	18.5±0.50	20.0±0.50	35	
AEAS	-	-	-	-	35	
JEAS	29.0±0.00	31.5±0.50[a]	32.0±0.00[a]	35.5±0.50[a]	35	*Escherichia coli*
EEAS	-	-	-	25.5±0.50[a]	35	
AEAS	15.5±0.50	16.0±0.00	19.0±0.00	20.5±0.50	35	

- = No zone of inhibition; Values are presented as Mean ± SEM, a=P < 0.05 compared to the standard drug (control) - One way ANOVA followed by Waller Duncan Post Hoc Test, Df=4 and N=15.

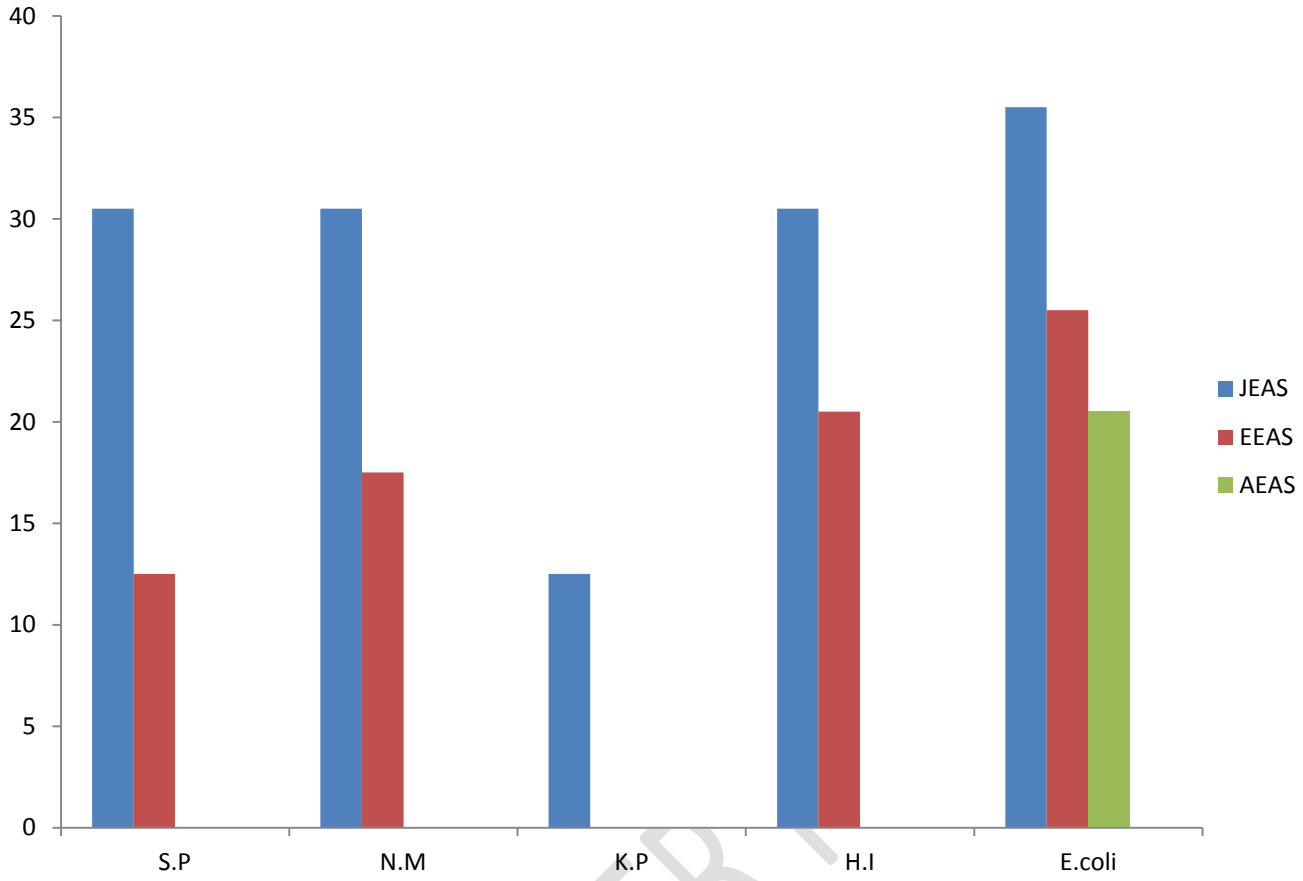

Figure 4: Minimum Inhibitory Concentration of Bulb extracts of *Allium sativum* JEAS=Juice Extract of *Allium sativum*, EEAS= Ethanolic Extract of *Allium sativum*, AEAS=Aqueous Extract of *Allium sativum*, S.P= *Streptococcus pneumoniae*, N.M= *Neisseria meningitides*, K.P= *Klebsiella pneumoniae*, H.I= *Haemaphilus influenzae*, E. coli= *Escherichia coli*.

3.4 Determination of Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC)

The result obtained shown below revealed the efficacies of *A. sativum* extracts' inhibitory effects, and the rate of killing the pathogenic bacteria isolates (Table 5). The time of killing of bacteria can easily be deduce to be the ratio of MBC/ MIC = 1 or more than 1, the rate of killing (KR) will be rapid, and is the potential weapon for eradicating or combating pathogenic bacteria and thereby reduce hemorrhagic sepsis with systemic dysfunctions [26].

Table 5: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of Standard Drug (Cefuroxime axetil) (5mg/ml)

Clinical Isolates + Cefuroxime axetil	Concentration of standard drug (cefuroxime axetil) in (mg/ml)												
	5	2.5	1.25	0.63	0.313	0.157	0.76	0.04	0.02	0.01	MIC Values	MBC Values	MBC/MIC =KR
S. pneumoniae	-	-	-	-	-	-	-	-	+	+	0.04	0.04	1.00
N. meningitides	-	-	-	-	-	-	-	-	+	+	0.04	0.76	19.00
K. pneumoniae	-	-	-	-	-	-	+	+	+	+	0.157	0.63	4.01
H. influenzae	-	-	-	-	-	-	-	-	+	+	0.04	0.157	4.00
E. coli	-	-	-	-	-	-	-	-	-	+	0.02	0.04	2.00

- = No viable growth, + = viable growth

Table 6: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (10mg/ml)

Clinical Isolates + Extracts		Concentration of *A. sativum* extracts in (mg/ml)												
		10	5	2.5	1.25	0.63	0.31	0.16	0.08	0.04	0.02	MIC Values	MBC Values	MBC/ MIC=KR
S. pneumoniae	JEAS	-	-	-	+	+	+	+	+	+	+	2.5	NB	
	EEAS	-	+	+	+	+	+	+	+	+	+	10	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
N. meningitides	JEAS	-	-	-	+	+	+	+	+	+	+	2.5	NB	
	EEAS	-	+	+	+	+	+	+	+	+	+	10	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
K. pneumoniae	JEAS											NA	NA	
	EEAS											NA	NA	
	AEAS											NA	NA	
H. influenzae	JEAS	-	-	+	+	+	+	+	+	+	+	5	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
E. coli	JEAS	-	-	-	-	-	+	+	+	+	+	0.63	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	-	-	+	+	+	+	+	+	+	+	5	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

Table 7: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (15mg/ml)

Clinical Isolates + Extracts		Concentration of *Allium sativum* extracts in (mg/ml)												
		15	7.5	3.8	1.88	0.94	0.47	0.23	0.12	0.06	0.03	MIC Values	MBC Values	MBC/ MIC=KR
S. pneumoniae	JEAS	-	-	-	-	+	+	+	+	+	+	1.88	NB	
	EEAS	-	+	+	+	+	+	+	+	+	+	15	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
N. meningitides	JEAS	-	-	-	-	-	+	+	+	+	+	0.94	NB	
	EEAS	-	+	+	+	+	+	+	+	+	+	15	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
K. pneumoniae	JEAS											NA	NA	
	EEAS											NA	NA	
	AEAS											NA	NA	
H. influenzae	JEAS	-	-	-	+	+	+	+	+	+	+	3.8	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
E. coli	JEAS	-	-	-	-	-	-	-	+	+	+	0.23	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	-	-	-	+	+	+	+	+	+	+	3.8	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

Table 8: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (20mg/ml)

Clinical Isolates + Extracts		Concentration of *Allium sativum* extracts in (Mg/ml)												
		20	10	5	2.5	1.25	0.63	0.313	0.16	0.08	0.04	MIC Values	MBC Values	MBC/ MIC=KR
	JEAS	-	-	-	-	-	+	+	+	+	+	1.25	2.5	2.00
S. pneumoniae	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	JEAS	-	-	-	-	-	-	+	+	+	+	0.63	1.25	2.00
N. meningitides	EEAS	-	+	+	+	+	+	+	+	+	+	20	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	JEAS											NA	NA	
K. pneumoniae	EEAS											NA	NA	
	AEAS											NA	NA	
	JEAS	-	-	-	-	+	+	+	+	+	+	2.5	5.0	2.00
H. influenzae	EEAS	-	-	-	+	+	+	+	+	+	+	5	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	JEAS	-	-	-	-	-	-	-	-	+	+	0.16	0.313	2.00
E. coli	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	-	-	-	-	+	+	+	+	+	+	2.5	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

Table 9: Minimum inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (25mg/ml)

Clinical Isolates + Extracts		Concentration of *Allium sativum* extracts in (mg/ml)												
		25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	MIC Values	MBC Values	MBC/ MIC=KR
	JEAS	-	-	-	-	-	-	+	+	+	+	0.78	1.56	2.00
S. Pneumoniae	EEAS	-	+	+	+	+	+	+	+	+	+	25	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	JEAS	-	-	-	-	-	-	-	+	+	+	0.39	0.78	2.00
N. meningitides	EEAS	-	+	+	+	+	+	+	+	+	+	25	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	JEAS	-	-	+	+	+	+	+	+	+	+	12.5	NB	
K. pneumoniae	EEAS											NA	NA	
	AEAS											NA	NA	
	JEAS	-	-	-	-	-	+	+	+	+	+	1.56	3.13	2.00
H. influenzae	EEAS	-	-	-	-	+	+	+	+	+	+	3.13	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	JEAS	-	-	-	-	-	-	-	-	-	+	0.10	0.20	2.00
E. coli	EEAS	-	-	-	-	-	+	+	+	+	+	1.56	NB	
	AEAS	-	-	-	-	-	-	-	+	+	+	0.39	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

CONCLUSION

The results of this study showed that bulbs extracts of *A. sativum* possessed active phytochemicals along with organosulphur volatile oil substances, which can be used as novel antibacterial agents. It exhibited potent antihemorrhgic and antibacterial activities which fight microbes due to its phyto constituents, antioxidant properties, and dietary medicines and coupled with zeolite herbominerals present. However (replace with Moreover), A. sativum extract can interact with the entire systems. It plays a vital role in human health, human nutrition as integrative medicine against bacterial meningitis pathogens. Therefore, there is need for further investigations in terms of toxicological studies and purification of bioactive components with a view to using *A. sativum* in novel drug development. This study has justified and validated the folkloric usage of *A. sativum* as local anti-infectious therapeutics in any bacterial bioterrorism (as natural disaster).

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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