ABSTRACT

The studies on phytochemical, nutraceutical profiles and potential medicinal values of *Allium sativum* Linn (Liliaceae) on bacterial meningitis were evaluated against bacterial meningitis pathogens. The methods employed in this study were validation of phytochemical screening which was done according to standard methods, determination of nutritional composition was carried out using analytical automated instruments (Atomic Absorption Spectrometers) and evaluation of in vitro antibacterial activities of the extracts against clinical isolates using agar-well diffusion and broth dilution methods. The clinical isolates of meningitis pathogens, *Streptococcus pneumoniae*, *Neisseria meningitides*, *Klebsiella pneumoniae*, *Haemophilus influenzae* and *Escherichia coli* were obtained from Ahmadu Bello University Teaching Hospital (ABUTH), Shika-Zaria. 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...
1. INTRODUCTION

Bacterial meningitis is a complication of malnutrition and a serious public health burden of high morbidity and mortality especially in the impoverished rural communities of Sub-Saharan Africa. The BM causes cellular hemorrhagic sepsis, metabolic, endocrinologic and neurologic disorders.

The Allium sativum bulb belongs to the family of Liliaceae formally known as Alliaceae and amongst the family, Allium sativum is the most popular food-herb used as condiment all over the world. The phytotherapeutic properties of garlic is caused by the combination of phytochemicals and biological activity of organosulfur such as allitrudium, allixin, S-allylcysteine, ajone, diallyl disulfide, alllicin organo-selenium compounds and garlicine. Researchers have been given garlic immense attention for 4-5 decades in its nutraceuticals and medicinal properties with wonderful health benefits due to high organo-sulfur compounds. This burden of the disease is highest in the developing countries and especially in the immunity compromised rural populations [1].

Malnutrition linked to BM is a great economic and societal burden in developing countries of Africa including Nigeria. As with most pathological disorders of the Sub-Saharan African countries often due to malnutrition that stems from poverty, consumption of foods deficient of vital nutrients unavailability and/or high cost of the right sorts of food especially in rural communities [1,2].

These medicinal and therapeutic properties are strongly hybridized by the combination of its secondary metabolites such as flavonoids and terpenoids and biological activities of its volatile organo-sulphur compounds. Garlic has high nutritional value and also highly valued in folkloric medicines all over the world. It has been found to contain active principles such as allicin, allistatin, ajocine, allitridium, allixin, S-allylcysteine, diallyl disulphide, allylmethylsulphate (AMS as pungent smell that causes garlicophobia), garcinin and scordinin which are of use in certain illnesses and conditions. A. sativum also contains sulphurous volatile oil (nigellone and eugenol), macrophytonutrients and Microphytonutrients, vitamin K and iodoform compounds.

Nutritional deficiencies or lack of vital nutrients in diets often leads to the development of certain physiologic and biochemical malfunctions that weakens the immune defense and predisposes the vital organs of the body to damaging infections. Meningococcal meningitis and its epidemics (cerebrospinal fever) have been
reported to have about 95% links to malnutrition [2]. BM is a serious bacterial illness characterized by the inflammation of any or all of the membranes (*dura mater*, arachnoid and *pia mater*) enclosing the central nervous system (brain and spinal cord) and which often leads to the damage of the blood vessels and meninges [3,4]. It is mostly endemic during the hot dry season typical of the tropical Sub-Saharan African countries, the hot climate of which favours the breeding of the meningococcal organisms that infect the meninges [5,6]. Inflammation of the meninges causes leakage of the infected cerebrospinal fluid (CSF) and alteration of the brain system (cognitive deficit) giving rise 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 etc).

Meningococcal-induced haemorrhage is a complication of malnutrition and a serious health burden of economic and societal problems especially in the impoverished rural communities of Sub-Saharan Africa and has actually been noted to be of high impact in the Northern States of Nigeria probably with regards to the exceptionally hot dry weather and climate of this part of the country [7,8,9].

Traditional medicine constitutes an important source of drugs for ethnopharmacological relevance and investigation. Various medicinal food-plants and animal products-supplements are available for use in certain immune-deficiency disease conditions related to malnutrition such as infectious disease and hemorrhagic sepsis. Furthermore, phytotherapy still remains a habitual part of health care system wholly or in part especially in rural communities [10,11]. *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 phytochemical and specific in vitro antibacterial activity on meningococcal bacterial organisms in condition of meningococcal-induced cellular injuries having been known to boost the immune system [12,13,14].

Crises of malnutrition in rural communities with its associated weakening of the immune system that leads to invasion of bacterial infections is a cause for concern that warrants a search for food plant-herb that can boost the immune system and help reduce spontaneous bursting of blood vessels, bleeding of blood vessels and bacteria-induced cellular injuries through adequate nutritional balanced diets ([15,2].

*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 meningococcal bacteria, nutritional values 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 as a biomedical basis.

As for the prevalence of meningococcal bacterial organisms, the potential of epidemic virulence of meningococcal bacteria organism in Nigeria varies with the geographical location, time of season, climate and pathogenic serotype strains of the causative organism (Lovera and Arbo, 2005). 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 [9]. The epidemics of Sudan in 2006 was of serotypes W-135 and X [16,17]. The most prevalent meningococcal 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 [18]. 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) [18,6]. Thus, these five bacterial organisms are those mostly known to be involved in meningitis, systemic septic-infections and hemorrhage in Sub-Saharan Africa including Nigeria [18]. 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 yrs have increased risk of *Escherichia coli* [3,4,19]. The aims of this research are to study the phytochemical, nutraceutical profiles and the potential medicinal values of *Allium sativum* L extracts of the fresh juice (JEAS), ethanolic (EEAS) and aqueous (AEAS) extracts on bacterial meningitis pathogens. To validate the phytochemical screening of the extracts of AS (JEAS, EEAS and AEAS). To investigate the proximate value and nutritional constituents of the extracts and its potential usage in folkloric medicine.

### 1.1 The Pathophysiology of Bacterial Meningitis

Usually the weakening or slacks in the functions of the immune defense system predisposes the body system to damaging opportunistic infections. Bacterial infection in particular often leads to septicaemia or persistent multiplication that traumatizes the blood vessels. The bacterial endotoxins tend to rupture its traumatized blood vessels and often cause bleeding and shunt in the flow of blood to the vital organs thus disrupting their functions (vital organs shot down) [20]. The endotoxin of both the gram negative contains coat with arabinoside peptidoglycan and lipopolysaccharide and gram positive coat with lipoteichoic acid and muramyldipeptide. *Neisseria meningitides* often get tangled with blood vessels resulting in their spontaneous bursting, or rupture of the blood vessel which can cause gushing out of blood (haemorrhage) accompanied with the weakness of collagen capacity [20]. There are some of the bacterial organisms that also have the tendency of producing clotting enzymes (coagulases) which down regulates the clotting mechanisms by forming coagulums (conversion of thrombin to fibrin) resulting in disseminated intravascular coagulation (DIC) of blood cells into thick solid mass (scabs) that impede blood flow and shuts down the vital organs [21]. The gram negatives of the bacterial organisms coagulate the blood faster and these coagulums may lead to stroke and failure of vital organs. Sometimes, the bleeding blood cells tend to form pus (haemorrhagic sepsis) and in union with circulatory hindrances and excessive tension on blood pressure. They produce generalized inflammations particularly encephalitis, arthritis, cellulitis, vasculitis, osteomyelitis etc), and other signs and symptoms of meningitis (meningism) such as stiffness especially of the neck. It gives rise to neuroinflammation in which the brain responds to infections, diseases and cellular injuries as post infectious attack to form meningitis associated paralysis (MAP) [20]. 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 [21,22]. 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 [22]. 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 [22]. 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, thus nutrition, disease and immune system are interrelated [22].

### 1.2 Aetiological Factors of Bacterial Meningitis

- Malnutrition
- Climate change
- 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 [23,2].
1.3 Description and Origin of *Allium sativum*

*A. sativum* (AS) is a food-plant originating from central Asia, but now grown everywhere in the world. For instance, this medicinal plant with bulb-stem resembling that of onion is now grown in Northern Nigeria. Garlic is the common name while *Allium sativum* is the scientific name. It belong to family of Liliaceae and locally called ‘tajarnuwa’ in Hausa, ‘allubosa ayu’ in Yoruba, ‘allibasa ayo’ in Ibo and ‘allivasa ahono’ in Ebira. It grows 2-3 feet in height and is annual or biennial. The name garlic is derived from the shape of the plant-leaf- ‘gar’ meaning spear; thus it is a spear-shaped leaf plant. Garlic bulb is available throughout the seasons and its flowers, leaves and bulbs are often used as culinary and so it is often cultivated as a cash crop. Garlic has high nutritional value and also highly valued in folkloric medicines all over the world. It has been found to contain active principles such as allicin, allistatin, ajocine, allitridium, allixin, garlicin and scordinin which are of use in certain illnesses and conditions [10,24]. *A. sativum* also contains sulphorous volatile oil, macrophytonutrients, vitamin K, flavonoids, terpenes and iodoform compounds. The *A. sativum* oil contains nigellone and eugenol often used for bronchial spasms [25].
1.4 Health Benefits of Garlic

- Strong flavored, garlic cloves contain many unique phytonutrients, minerals, vitamins, and antioxidants that have proven health benefits. Total measured antioxidant strength (ORAC value) is 5346 μmol TE/100 g.
- Its bulbs contain organic thiosulfinate compounds such as diallyl disulfide, diallyl trisulfide and allyl propyl disulfide. Upon disruption of the bulb (while crushing, cutting, etc.), these compounds convert into allicin through an enzymatic reaction.
- Laboratory studies show that allicin reduces cholesterol production by inhibiting the HMG-CoA reductase enzyme within the liver cells.
- Allicin decreases blood vessel stiffness through the facilitation of nitric oxide (NO) release. Nitric oxide relaxes blood vessels and thereby, brings a reduction in the total blood pressure. Further, it blocks platelet clot formation and has fibrinolytic action inside the blood vessels. This function of allicin helps decrease the overall risk of coronary artery disease (CAD), peripheral vascular diseases (PVD), and stroke.
- Research studies also suggest that consumption of garlic associated with a possible decrease in the incidence of stomach cancer.
- Allicin and other essential volatile compounds also found to have antibacterial, antiviral, and anti-fungal activities [24].

Garlic is an excellent source of minerals and vitamins that are essential for optimum health. The bulbs are one of the richest sources of potassium, iron, calcium, magnesium, manganese, zinc, and selenium. Selenium is a heart-healthy mineral and is an essential cofactor for antioxidant enzymes within the body. The human body uses manganese as a co-factor for the antioxidant enzyme, superoxide dismutase. Iron is essential for red blood cell formation.

It contains many flavonoid antioxidants like β-carotene, zeaxanthin, and vitamins like vitamin-C. Vitamin-C helps the body develop resistance against infectious agents and scavenge harmful, pro-inflammatory free radicals [24].

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 [10,26]. It has also been reported to have antitumour and anticholesterolaemia properties whereby it is used to decrease low density lipoprotein and 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) [26,27].

2. MATERIALS AND METHODS

2.1 Equipments and Instruments

Spectronic 21D/flame photometer (Unicam 969 AAS; Milton Roy Co., Rochester, New York)

Fast Sequential Atomic Absorption Spectrometer (Varian AA240FS; Amalgamated Biotech, USA)

Spectrophotometer (Buck 205 AA; Amersham Biosciences, USA)

Bacteria incubator (Dapco Model 630, USA)

Autoclave (Adelphi Co., USA)

Oven (Tatlock, 414462/27, UK)

Water bath (Gallenkamp, UK)

Electronic Weighing Balance (Mettler P162, UK) – (used for weighing extracts)

Electronic Weighing Balance (Ohaus Champ 11, UK) – (used for weighing animals)

2.2 Drugs, Solvents and Other Materials

Cefuroxime axetil (Cefunat, USP; BE207, Evans Medical Plc Lagos, Nigeria)

Ethanol (96%) (Sigma-Aldrich, USA).

Mueller-Hinton Agar Media (Liofilchem, Italy)

Nutrient Broth Media (Maharashtra, India)

Tween 80 (Sigma-Aldrich, USA)

Petri-dishes, Test-tubes, racks, forceps, wire loop, bend glass rod, filter paper, Mortar and pestle, electric blender, beaker, measuring cylinder.

2.3 Collection and Identification of Plant Material

The bulbs of *A. sativum* were obtained as fresh farm products from farmers at Sabon-Gari, Zaria, in Kaduna State, Nigeria in November, 2012. 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.

2.4 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. [28]. 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°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

2.5 Solutions

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.

2.6 Phytochemical Screening of *Allium sativum*

The methods of Trease and Evans [29] and Sofowora [11] 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. 2.0 g of powders of each
of the three extracts were dissolved in 100 ml of distilled water for this screening experiment.

The tests carried out include:

i. **Mayer’s test**: Extract solution (1 ml) + 1 ml of Mayer’s reagent added drop by drop that gives cream color precipitate indicates the presence of alkaloids.

ii. **Frothing test**: Extract solution (2 ml) + 5 ml distilled water shaken vigorously; was observed for 15 minutes for a honey comb froth formation that indicates presence of saponins.

iii. **Shinoda test**: Extract (0.1 g) + 2 ml of 5% ethanol + few magnesium chips + few drops of HCl; that forms a pink or red colour indicates the presence of flavonoids.

iv. **Molish test**: Five drops of Molish reagent + 2 ml of extract solution + 2 ml of concentrated H$_2$SO$_4$; that gives reddish ring indicate the presence of carbohydrates.

v. **Keller-Killiani test**: Glacial acetic acid (2 ml) + 1 drop of FeCl$_3$ + 1 ml of extract solution + 2 ml of concentrated H$_2$SO$_4$; that gives a purple-brown ring indicate presence of cardiac glycosides.

vi. **Ferric chloride test**: Extract solution (3 ml) + 1 ml of 5% FeCl$_3$ (in 3 drops); that gives a deep-green color indicate the presence of tannins.

vii. **Borntrager’s test**: Extract solution (1 ml) + 2 ml of chloroform was shaken for 5 minutes and filtered, then filtrate + 10% NH$_4$OH; solution that gives a rose pink colour indicate the presence of anthraquinones.

viii. **Test for fats and oils**: Filter paper soaked in the extract solution or impregnated with extract was allowed to dry and checked for translucence films; that indicate the presence of fat and oils.

**Liebermann-Buchard test**: Extract (1 ml) + 1 ml of acetic acid anhydride was mixed gently + 1 ml of concentrated H$_2$SO$_4$; that gives a blue-green color indicates presence of steroids (ring) and terpenoids.

### 2.7 Nutraceutical Profile of Garlic (*Allium sativum* Linn) as Nutritional Analysis

“Use one stone to kill two birds, Let food herb is your medicine and medicine is your food herb”. Currently there is an increased global interest due to the recognition that nutraceuticals play a major role in health enhancement. The term "Nutraceutical" was coined by combining the terms "Nutrition" and "Pharmaceutical" in 1989 by Dr Stephen De Felice, Chairman of the Foundation for Innovation in Medicine. Nutraceutical is a marketing term developed for nutritional supplement that is sold with the intent to treat or prevent disease and thus has no regulatory definition. Hence a nutraceutical is any substance that may be considered a food or part of a food and provides medical or health benefits, encompassing, prevention and treatment of diseases. Such products may range from isolated nutrients, dietary supplements and diets to genetically engineered designer foods, herbal products and processed foods such as cereals, soups and beverages. Presently over 470 nutraceutical and functional food products are available with documented health benefits [30,31].

Nutritional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates necessary for healthy survival. When functional food aids in the prevention and/or treatment of disease/disorder other than deficiency conditions like anemia it is called a nutraceutical. Thus, a functional food for one consumer can act as a nutraceutical for another [32]. Examples of nutraceuticals include pineapple (juice as such is a nutrient and its constituent bromelain is a pharmaceutical) and citrus fruits (orange juice is nutrient and its constituent ascorbic acid is a pharmaceutical) [33]. A dietary supplement is a product that is intended to supplement the diet that bears or contains one or more ingredients like, vitamins, minerals, herbs, amino acids, fatty acids or a concentrates, metabolites, constituents, extract, or combinations of these either. Medical foods are a specific category of therapeutic agents that are intended for the nutritional management of a specific disease. An example of medical foods is formulations intended to manage patients with inborn errors in amino acid metabolism. Such as garlic is one of the food herbs that have phytotherapeutic potential in the management of diseases [33, 30,31].

### 2.8 Analysis of Nutritional Composition and Proximate Values of *Allium sativum* Extract (JEAS)

Analytical automated instruments including spectronic 21D / flame photometer, unicum 969 AA spectrometer (AAS), fast sequential atomic spectrometer AA240FS and AA spectrophotometer Buck205 were used to analyze for
both nutritional composition and their proximate values in the JEAS according to the methods described by Fahey [15]; Naturopathy [34] and Van [35]. 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°C and filtered to obtain the filtrate for the analytical studies for A. sativum nutritional composition and 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. 2.0 g of JEAS powder was dissolved in 100 ml of DH₂O for the quantitative determination of vitamins, macro- and micro-elements and other nutrients. 10ml for vitamin analysis (200 mg), 30 ml for macro/micro (zeolite) element analysis (600 mg) and 30 ml for other nutrients analysis (600 mg) respectively.

2.9 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°C for 24 hours as described by WHO [36] 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 1 ml was removed into the third tube which was then deemed to be 1x10³ 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 1x10⁵ CFU/ml. The last diluted concentrations (5 test tubes of the bacterial organisms) were incubated at 37°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, [37]. Four different concentrations (10, 15, 20 and 25 mg/ml) and 5 mg/ml of the standard drug (cefuroxime) 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 of 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 [38,36].

2.10 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 1 ml 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°C for 24 hrs. 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 [38]. 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°C for 24 hours and checked for growth inhibition [38,37].

3. RESULTS

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.

3.2 Phytochemical Screening of the Extract

The Table 2 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>
<thead>
<tr>
<th>Extract</th>
<th>% Yield (w/w)</th>
<th>Observed colouration</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEAS</td>
<td>12.7</td>
<td>light yellow powder</td>
</tr>
<tr>
<td>EEAS</td>
<td>17.2</td>
<td>light yellow powder</td>
</tr>
<tr>
<td>AEAS</td>
<td>14.7</td>
<td>Brown powder</td>
</tr>
</tbody>
</table>

Note: JEAS= juice extract of Allium sativum, EEAS= ethanolic extract of Allium sativum and AEAS= aqueous extract of Allium sativum

<table>
<thead>
<tr>
<th>Phytochemical components</th>
<th>JEAS</th>
<th>EEAS</th>
<th>AEAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac /glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fats and Oils</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroidal ring</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: JEAS= juice extract of Allium sativum, EEAS= ethanolic extract of Allium sativum and AEAS= aqueous extract of Allium sativum. Note: + = Present and - = Absent.
3.3 The Nutritional Composition and Proximate Values in the JEAS Extract

From the analysis carried out for nutritional composition, nutrients such as carbohydrate, dietary fibre, protein, fat, vitamins, macro- and micro-herbominerals with their proximate values were observed as summarized in Table 3.

3.4 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 30 mg/ml showed significant inhibitory P<0.05 in in-vitro susceptibility test.

3.5 Undesirable Effects

The sulfide compounds in the garlic metabolized to allyl methyl sulfide (AMS), which is excreted through sweat due to rapid garlikokinetivity and breathe producing unpleasant odour and breath (halitosis). But act as snake and mosquito repeller.

4. DISCUSSION

*Allium sativum* extracts were found to express high antibacterial activities on *Escherichia coli*, *Helicobacter pylori*, *Salmonella hadar*, *Neisseria meningitides*, *Streptococcus pneumoniae*, *Salmonella typhi* and Enterobacteriaceae, in which *E. coli* and *Salmonella typhi* are the most common of gastrointestinal infections [10,28]. About 80% of the human populations in Nigeria are long-time carriers of these bacterial species which are highly virulent in neonates, growing infants and young adults [19,6] and are responsible for infections like ulcer, dysentery, septicaemia and typhoid fever. These Gram-negative bacteria can also cause life-threatening diseases such as otitis media, pneumonia, bacterial meningitis, endocarditis, chest pain, respiratory distress and bacterial hemorrhagic sepsis [18,22].

The aqueous extract of *A. sativum* was evaporated over a water bath at 50°C to obtain AEAS powder, which was brown in colour and had low biologic activities. It may be due to heat applied via water bath and this led to loss of some of its bioactive constituents that heat labile. Similar activity was observed and reported by Greiner and Konielzny [39]. JEAS powder and EEAS powder showed significant high biologic activities. Different solvents yielded substantial differences in the proportion of extracts obtained. The differences in the percentage yields might be due to the polarity of the solvents, the less polar solvents such as ethanol, yielding greater quantities of the extracts compared with the more polar solvents such as water (Table 1). JEAS was the least in percentage yield but more potent and efficacious in biologic activities than EEAS and AEAS (Table 2, Table 3 and Table 4).

<table>
<thead>
<tr>
<th>Nutritional components</th>
<th>Vitamins</th>
<th>Proximate values (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vit A (β-Carotene)</td>
<td>14.00</td>
</tr>
<tr>
<td></td>
<td>Vit B&lt;sub&gt;1&lt;/sub&gt; (Thiamine)</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>Vit B&lt;sub&gt;2&lt;/sub&gt; (Riboflavin)</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Vit B&lt;sub&gt;3&lt;/sub&gt; (Niacin)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Vit B&lt;sub&gt;5&lt;/sub&gt; (Pantothenic acid)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Vit B&lt;sub&gt;6&lt;/sub&gt; (Pyridoxine)</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>Vit B&lt;sub&gt;9&lt;/sub&gt; (Folic acid)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Vit H (Biotin)</td>
<td>95.00</td>
</tr>
<tr>
<td></td>
<td>Vit B&lt;sub&gt;12&lt;/sub&gt; (Cyanocobalamin)</td>
<td>8.22</td>
</tr>
<tr>
<td></td>
<td>Vit C (Ascorbic acid)</td>
<td>21.40</td>
</tr>
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<td></td>
<td>Vit D (Ergocalciferol)</td>
<td>4.83</td>
</tr>
<tr>
<td></td>
<td>Vit E (α-Tocopherol)</td>
<td>31.20</td>
</tr>
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<td></td>
<td>Vit K (Phytonadione)</td>
<td>2.16</td>
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<table>
<thead>
<tr>
<th>Macro/micro (Zeolite) elements</th>
<th>Proximate values (mg)</th>
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<tbody>
<tr>
<td>Sodium</td>
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<tr>
<td>Potassium</td>
<td>154.00</td>
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<tr>
<td>Calcium</td>
<td>184.00</td>
</tr>
<tr>
<td>Magnesium</td>
<td>26.00</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>390.00</td>
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<tr>
<td>Sulphur</td>
<td>450.00</td>
</tr>
<tr>
<td>Iron</td>
<td>1.82</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.03</td>
</tr>
<tr>
<td>Silver</td>
<td>0.01</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.05</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.18</td>
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<tr>
<td>Selenium</td>
<td>0.014</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Other nutrients</th>
<th>Proximate values (mg)</th>
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</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>54.39</td>
</tr>
<tr>
<td>Dietary Fibre</td>
<td>2.20</td>
</tr>
<tr>
<td>Protein</td>
<td>32.40</td>
</tr>
<tr>
<td>Fat</td>
<td>4.00</td>
</tr>
</tbody>
</table>
are known to form complexes with volatile sulphurous oil and flavonoids in this plant nervous system pathologic responses in the systemic and central antidropsy effect and reduced inflамmation demonstrated activity against bacteria; have terpenoids were also reported to have saponins, cardiac glycosides, oils, fats and antibacterial activities synthesized by plant in response to microbial volatile oils have been reported to be parasites and viruses; flavonoids and sulphurous various organisms such as bacteria, fungi, possess appreciable inhibitory activities (Table 2). Some of these compounds were reported to possess antimicrobial activities (Table 2). Some of these compounds are responsible for the characteristic odours like allylmethylsulphate (AMS) as garlicophobia, pungencies and colour of the plants while others give a particular plant its culinary, medicinal or poisonous virtue [29,11]. From the result of the phytochemical screening the extracts were found to contain saponins, fats and oils, flavonoids, alkaloids, carbohydrates, cardiac glycosides and terpenoids. In previous studies that were also carried out, the same phytochemical constituents were reported to possess antimicrobial activities and effects on heart [10,40]. The secondary metabolites have been variously reported to possess appreciable inhibitory activities against various organisms such as bacteria, fungi, parasites and viruses; flavonoids and sulphurous volatile oils have been reported to be synthesized by plant in response to microbial infection and have been shown to have antibacterial activities [41,10]. Alkaloids, saponins, cardiac glycosides, oils, fats and terpenoids were also reported to have demonstrated activity against bacteria; have antitrospy effect and reduced inflamмatory pathologic responses in the systemic and central nervous system [10,27]. Alkaloid, saponins, volatile sulphurous oil and flavonoids in this plant are known to form complexes with peptidoglycans, sterols and other cell wall components of bacteria resulting to cell leakage, cellular apoptosis and finally death of bacteria [28,42].

The phytochemical screening of the bulb extracts (JEAS, EEAS and AEAS) for bioactive constituents observed in A. sativum extracts revealed phytoconstituents with biological activities (Table 2). Some of these compounds are responsible for the characteristic odours like allylmethylsulphate (AMS) as garlicophobia, pungencies and colour of the plants while others give a particular plant its culinary, medicinal or poisonous virtue [29,11]. From the result of the phytochemical screening the extracts were found to contain saponins, fats and oils, flavonoids, alkaloids, carbohydrates, cardiac glycosides and terpenoids. In previous studies that were also carried out, the same phytochemical constituents were reported to possess antimicrobial activities and effects on heart [10,40]. The secondary metabolites have been variously reported to possess appreciable inhibitory activities against various organisms such as bacteria, fungi, parasites and viruses; flavonoids and sulphurous volatile oils have been reported to be synthesized by plant in response to microbial infection and have been shown to have antibacterial activities [41,10]. Alkaloids, saponins, cardiac glycosides, oils, fats and terpenoids were also reported to have demonstrated activity against bacteria; have antitrospy effect and reduced inflamмatory pathologic responses in the systemic and central nervous system [10,27]. Alkaloid, saponins, volatile sulphurous oil and flavonoids in this plant are known to form complexes with peptidoglycans, sterols and other cell wall components of bacteria resulting to cell leakage, cellular apoptosis and finally death of bacteria [28,42].

Table 3 shows the nutritional composition and proximate values of the JEAS of A. sativum. The result showed the presence of carbohydrates, dietary fibres, proteins, fats, vitamins and macro- and micro-elements (zeolite herbominerals) as primary metabolites. Thus, this plant extract contained all classes of food nutrients, a complete nutritious and balanced diet as nutritional utility plays a vital role in biocidal or bactericidal effect on these pathogenic bacteria. In fact, the presence of all food nutrients in A. sativum bulb extract is an indication that it contains a natural vital nutritional source for health maintenance, blood interactor; prevents and boosts the body immune system against diseases. Both the primary and secondary metabolites of this plant, work best in combination by supporting each other as integrative medicines [15,27,25]. In addition, nutritional and medicinal properties of A. sativum extract is said to be pharmacologically active through zeolite- chelating, antioxidant and ameliorative properties. The findings observed were antioxidant properties, chelation effects and nanopharmacologic of Silver, Manganese, Zinc, Iron and Selenium; which exerts biocidal properties as well as stimulates immune system to cushioning the challenges of the BM

<table>
<thead>
<tr>
<th>Zone of growth inhibition (mm) of the extracts at drug concentrations (mg/ml)</th>
<th>Test extracts</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>5 (cefuroxime)</th>
<th>Bacterial organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEAS</td>
<td>20.5±0.50</td>
<td>25.5±0.50</td>
<td>29.5±0.50</td>
<td>30.5±0.50</td>
<td>35</td>
<td>Streptococcus pneumoniae</td>
<td></td>
</tr>
<tr>
<td>EEAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.5±0.50</td>
<td>35</td>
<td>Neisseria meningitides</td>
<td></td>
</tr>
<tr>
<td>AEAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>35</td>
<td>Klebsiella pneumonia</td>
<td></td>
</tr>
<tr>
<td>JEAS</td>
<td>28.0±0.00</td>
<td>33.5±0.50</td>
<td>34.0±0.00</td>
<td>36.0±0.00</td>
<td>35</td>
<td>Haemophilus influenzae</td>
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<tr>
<td>EEAS</td>
<td>-</td>
<td>14.5±0.50</td>
<td>17.5±0.50</td>
<td>-</td>
<td>35</td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>AEAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JEAS</td>
<td>23.0±0.00</td>
<td>26.5±0.50</td>
<td>28.0±0.00</td>
<td>30.5±0.50</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEAS</td>
<td>-</td>
<td>15.5±0.50</td>
<td>18.5±0.50</td>
<td>20.0±0.00</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>35</td>
<td></td>
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</tr>
<tr>
<td>JEAS</td>
<td>29.0±0.00</td>
<td>31.5±0.50</td>
<td>32.0±0.00</td>
<td>35.5±0.50</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEAS</td>
<td>15.5±0.50</td>
<td>16.0±0.00</td>
<td>19.0±0.00</td>
<td>20.5±0.50</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- = 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
pathogens, its potency of this plant is engineered by nature [39,43,25].

The presence in A. sativum of phytoconstituents, which have medicinal properties, dietary supplements and food ingredients (Table 2 and 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 [43]. It protects entire body by stimulating the immune system, prevents infectious diseases, improves health, and supports the structures (collagens) [15,35,27]. Pharmacologically, the JEAS extract has demonstrated an interesting pattern of bacterial susceptibility against clinical isolates tested. Its MBC values were higher than the MIC values, indicating that the bactericidal effect of the JEAS could result to bacterial eradication.

It was reported that the bacterial killing rate of an extract whose ratio of MBC to MIC is closer to one (1) rate of time, and will be rapid and a potential weapon against pathogenic bacteria eradication [42].

5. CONCLUSION

This study has succeeded in providing valuable scientific evidence of good nutritional profiles and potent antibacterial activity as a natural antibiotic and biodefensive agent. This experimental investigation has provided the scientific validation for the ethnomedical use of A. sativum as a remedy to treat bacterial meningitis locally.

CONSENS AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

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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.

REFERENCES


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