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Essential oil, antioxidant properties of silver nanoparticles and methanol extract of *Allium sativum.*

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Abstract
The bioactive compounds of Allium sativum was carried out using essential oil from the spice. The SEM shows the shapes, dispersion and agglomeration of the sample, while the EDX confirms the SEM and the presence of some compounds; Ag L (Silver iodide), Ci K (Potassium chloride), C K (Cyanogen chloride), P K (Phenol), S K (Potassium). The FT-IR reveal the AgNPS. capping and reducing the particular biomolecule from the functional group for identification. The 11 bioactive compounds of Allium sativum viewed on the GC-MS has shown to have different antioxidant role they play in human health and body. The DPPH of AgNPs Allium sativum was higher at 500µg/ml and has a concentration dependency than themethanol Allium sativum, but Allium sativum methanol is higher than the AgNPs as represented in the IC50 table. The reducing power of Allium sativum methanol was higher than the Allium sativum AgNPS. Therefore, Allium sativum can be beneficial in nutraceuticals industry.

Keyword; *Scanning Electron Microscope / Energy Dispersive X-Ray (SEM/EDX), Silver Nanoparticles (AgNPs), Fourier-Transform Infrared (FT-IR), Spectroscopy Gas Chromatography–Mass Spectrometry (GC-MS), Allium sativum (A.S), 2, 2-diphenyl-1-picryl-hydrazyl radical scavenging (DPPH), Silver Nitrate(AgNO3).*

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I. Introduction

Nanotechnology is an emerging field, which utilizes nanoparticles (NPs) in various applications such as infood packaging, as preservatives, in cosmetics, as carriers of therapeutic agents in nanomedicine (Shalaby*et al*., 2015; Alsammarraie *et al.*, 2018; Khan *et al.*, 2019). NPs are currently being exploited in the treatment of infectious diseases; they are regarded as a bridge between atomic structures and large sizes of materials (Alsammarraie *et al.*, 2018). The structural design of NPs is simple and come in different sizes ranging between 1 - 100 nm. Their peculiar features such as high energy on their surfaces together with a large surface area to mass ratio makes NPs effective in any given reaction, they are involved in. NPs also exhibit new specific properties in their particle distribution, size and shape (Mahardika *et al*., 2021). Nanoparticles that are inorganic in nature are unique and provide different functions to users (Alsammarraie *et al.*, 2018). Due to high efficiency in catalysis, biosensing and optics has made gold (Au) and silver (Ag)

NPs to be exploited in nanomedicine (Bouqellah *et al.*, 2019). However, silver nanoparticles (AgNPs) have exhibited promising potentials when employed in chemical reactions as well as excellent carriers of antioxidants and antimicrobial substances (Alsammarraie *et al*., 2018; Maghimaa and Alharbib, 2020).

Various methods have been employed in the synthesis of AgNPs, they include chemical, sonochemical, microwave, ultrasonification, irradiation and green processes (Chung *et al.*, 2016). All the aforementioned methods have harmful effects to the environment except green synthesis. They are also expensive and utilizes harmful chemicals in their synthesis (Shalaby *et al*., 2015; El-Deeb *et al*., 2016). As a result of this,green synthesis is always preferable since it is safe, cost effective, efficient, does not utilize high pressure and temperature (Maghimaa and Alharbi, 2020). The use of medicinal plants and spices among varying methods employed in green synthesis of AgNPs have proven to be suitable in the formation of stable AgNPswithin a short period of time due to the abundant phytochemicals they possess (Bashir *et al*., 2015) Medicinal plants are the backbone of modern medicine, their use in medicine have been known and utilized for centuries. Medicinal plants have taken lead roles as sources of important bioactive compounds to tackle infections caused by MDR bacteria (Otunola *et al*., 2017). However, these herbal drugs could sometimesencounter problems in the delivery system, thus, become

inefficient in the treatment of microbial infections. Some of these hindrances include low bioavailability, instability in biological, poor permeability and solubility among many other factors. These hindrances can be overcome when bioactive compounds in herbal drugs are encapsulated or attached to NPs, which enhances significantly the pharmacokinetics and the overall performance of the herbal drug. Medicinal plants are of great importance in green synthesis of NPs. They can control the shape and size of NPs depending on the phytochemicals they possess, which actas a capping layer. Different medicinal plants as well as spices have been employed in the production ofAgNPs (Otunola *et al*., 2017).

Allium sativum are common to most cuisines across the globe. Aside the culinary spice benefits, they also confer medicinal benefits and have been used by consumers as remedies for various diseases (Andleeb *et al*., 2020). Varying biological functions attributed to these spices include anticancer, hypoglycemic, antidiabetic, antihypertensive, immunomodulatory, hypolipidemic, antioxidant and antimicrobial among many others. The phytochemical investigation of these spices shows that they are rich in tannins, phenols, alkaloids, flavonoids, saponins and carotenoids, which have been demonstrated to exhibit strong antioxidant properties and can be considered as a reducing factor in green synthesis of AgNPs (Dinda *et al*., 2019; Sharma *et al*., 2020). As such, this study was aimed at evaluating the antibacterial efficacy of five spices synthesized with AgNPs against some selected pathogens.

Materials and MethodsCollection of spices

Allium sativum were purchased at Garki market Abuja. These spices were identified by a taxonomist from the Herbarium Unit of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja.

Raw Materials Preparation

The different samples were subjected to post-harvest treatment before experimental use. The modified method described by Adewole *et al,* (2013) was used accordingly. The particle size of the sample was determined manually by sieve analysis (Jillavenkatesa *et al*, 2001). The sample was sorted and placed in an air tight container for experimental analysis.

Extractions

- **Maceration of Methanol Extract**

Cold maceration was the method of extractions for the methanol extract. The solvent used was methanol (64.7^{o}C, the volume of the solvent was twice the physical size of the extract, the sample was crushed put in a big conical flask, the solvent added, covered and kept at a room temperature (20-25^{o}C of 24 hours, shaken at intervals. After maceration, the plant sample was filtered using muslin, the extract was put in a rotary- evaporator to reduce the volume in the extractant after which it was transferred into a stainless plate and put in a water bath (100^{o}C) for complete drying and methanol evaporation. The dried extract was collected(using a spatula) and put in an air-tight bottle container, kept in a cool dry place for laboratory analysis.

A.S *(Allium Sativum)* Quantity of sample - 1000g Quantity of solvent - 2000mlDays of drying – 4 days

- **Hydro-distillation - Essential Oil**

The essential oil was carried using hydro-distillation process. The plant sample was subjected to post-harvest treatment (figure 1) put in a round bottom flask, water was added (the water was almost the size of each sample in the flask), then set up in a glavenger and switched on. The extraction started, 0.5 ml of m- hexane was added into the pipe, where there was a mixture of water and oil to trap the oil. The water heating up and escaping within different pipes together with the essential oil. In the cylinder of the glavenger, water and oil mix (which comes out from the sample), the water was down while the oil was up, and before the process on, little water was added through a small opening up and a small wool was used to cover the opening so that the oil will not escape and the water added helped to cool the cylinder when there was abnormal increase in temperature during the heating process. As the oil extract and drop through the pipe, the oil is collected into a bottle, until the process is complete in about 2-3 hours. Using a syringe to remove the little water in the oil, then the volume of the essential oil is taken, the oil was put in an amber bottle, kept in the NIPRD laboratory fridge (7^{0}C) for GC-MS analysis.

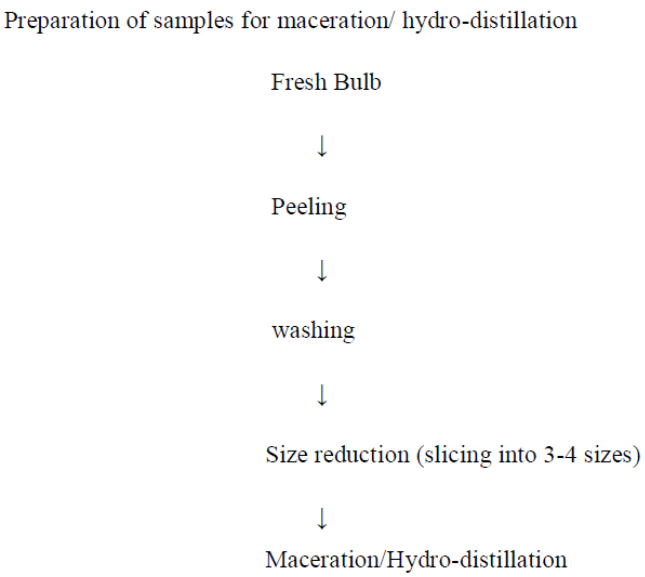


Figure 1: Flow diagram for processing of plant sample for Maceration / Hydro-distillationPreparation and synthesis of silver nanoparticles

AgNPs of each extract was synthesized by adopting the method described by Gloria *et al.*, (2017), twenty grams of *A. sativum* was weighed into individual 250 mL conical flasks and was extracted with 100 mL of deionized water at 60°C for 10 min in a water bath. The extracts were cooled and filtered using Whatman filter paper no. 1 under vacuum. Exactly 15 mL of the prepared extracts was added to 45 mL of aqueous AgNO3 (0.1 M solution) at room temperature and stirred continuously with a magnetic stirrer for 15 min. The solution obtained was kept in dark to prevent auto-oxidation of silver (Krenn *et al*., 2001).

Characterization of silver nanoparticles

After 24 hours, the solution containing AgNPs was centrifuged at 3000 rpm for 10 min, the resulting pellets was dried in an oven at 100°C for 24 hours. The purified AgNPs was characterized using the following techniques. The formation of AgNPs was monitored by visual assessment of the colour changes of the solutions. The reduction of silver was measured periodically at a wavelength range of 300–700 nm using a UV-Vis spectrophotometer (UV-3000 PC, UK). The UV-Vis spectra of AgNPs produced was plotted and recorded as a function of bio reduction time (15 min intervals) at room temperature at a resolution of 0.5 nm. Size, shape, and morphology of the nanoparticles was determined by scanning electron microscopy (SEM) (ZEISS prdt, Evo/LS10), the samples for SEM assays were sonicated for 5 min to make a suspension of AgNPs in distilled water. A drop of the suspension was then placed on double-sided-coated carbon stubs, allowed to dry, and observed using SEM at a voltage of 15–20 kV at different magnifications. Fourier Transform Infrared (FTIR) (Nicolet iS5, Thermo-scientific Berlin Germany). Analysis was used to determine the possible biomolecules responsible for the reduction of silver ions to AgNPs. The samples were analysed using a spectrometer. Spectra was collected from 50 scans at a resolution of 4 cm−1 in the range of 500-4000. The remaining pellet was used for AgNPs Antioxidant in comparism with Methanol extract.

Figure 2A

Figure 2B

Figure 2A showing the extract of un-synthesized sample and Figure 2B showing the synthesized AgNPS. *Allium sativum* (AS).

Antioxidant Assay

The antioxidant activities of the spice's methanol extract and AgNPs was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Reducing power. By Cheng and Yuanzong (2004).

Free Radical Scavenging Assays

2, 2-diphenyl-1-picryl-hydrazyl radical scavenging (DPPH) Assay. The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based onthe reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH. this transformation results in a colour change from purple to yellow, which is measured by a spectrophotometer. The disappearance of the purple colour is monitored at517 nm. The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl. The reaction mixture consists of 0.1 ml of DPPH in methanol (0.3mM), 1.0ml of the extract and 1.0ml of methanol. It is incubated for 10min in dark, and then the absorbance is measured at 517nm. In this assay, the positive controls were ascorbic acid. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition } \% = \frac{AU - A1}{AO} \; X\; 100$$

Where AO is the absorbance of control and A1 is the absorbance of test.

Reducing Power Assay (RP)

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of liquid per oxidation processes, so that they can act as primary and secondary antioxidants.

The reducing power was determined by taking 1.0 ml of extract with 2.5 ml of phosphate buffer (200 Mm,pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50^0C for 20min. thereafter, 2.5 mlof trichloroacetic acid (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (6 mM) and absorbance is measured at 700 nm. Ascorbic acid was used as positive control.

Gas Chromatography–Mass Spectrometry (GC-MS) analyses

The bioactive essential oil was analysed by gas chromatography–mass spectrometry (GC-MS) using Shimadzu QP-2010 GC with QP-2010 SE Mass Selective Detector [MSD], operated in the EI mode (electron energy=70 eV), scan range of 45-700 amu, and scan rate of 3.99 scans/sec], and Shimadzu GC- MS solution data

system. The Gas chromatography column was Optima-5MS fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 3.22 mL/min. The program used for Gas chromatography oven temperature was at 60°C held for 2 minutes, followed by 60-260°C at a rate of 13°C/min, then held at 260°C for 2.5 min. The injection port temperature was 250°C, ion source temperature 230 °C, while the interface temperature was 250°C. Diluted sample (1/100 in hexane, v/v) of
1.0 µL was injected using autosampler and in the split mode with ratio of 10:1. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentages of each component are reported as raw percentages based on the total ion current.

II. Results

Scanning Electron Microscope / Energy Dispersive X-Ray

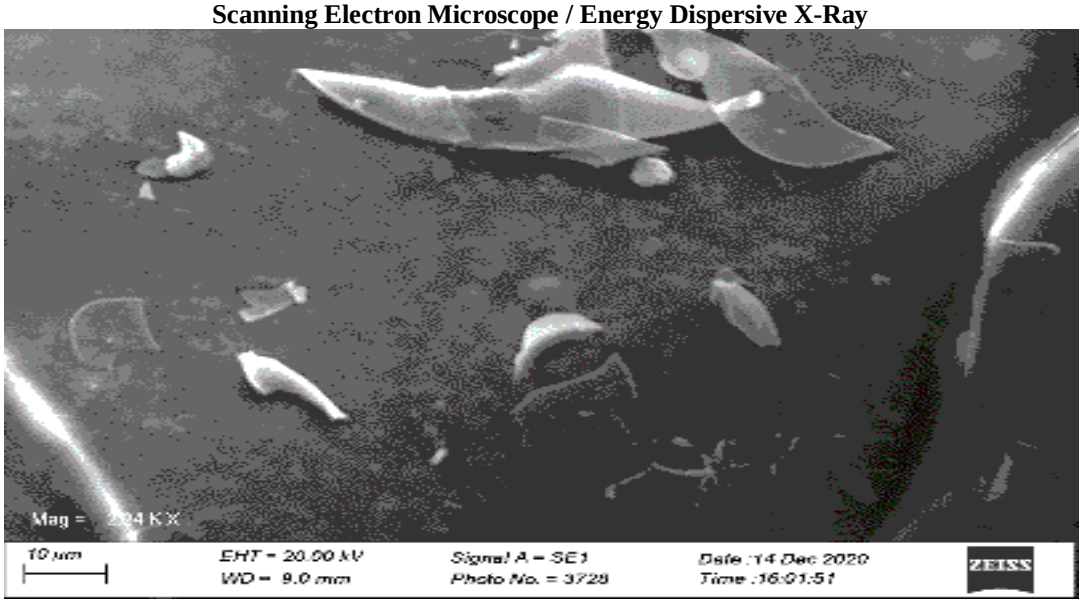


Figure 3a: SEM of *Allium Sativum*

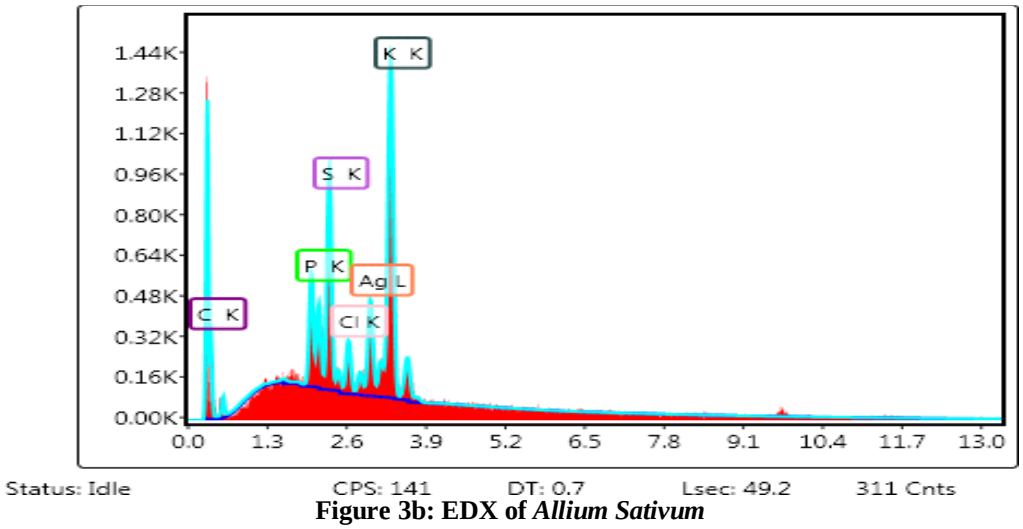


Figure 3b: EDX of *Allium Sativum*

Fourier-Transform Infrared Spectroscopy

The absorbance of the sample as a function of wave number (cm^{-1}) was determined using FTIR (Nicolet iS5, Thermo-scientific Berlin Germany). The FTIR was carried out to identify the functional groups and the types of bonds occurring at the range of 500-4000 (cm^{-1}). Figure below presents the FTIR spectra of AS

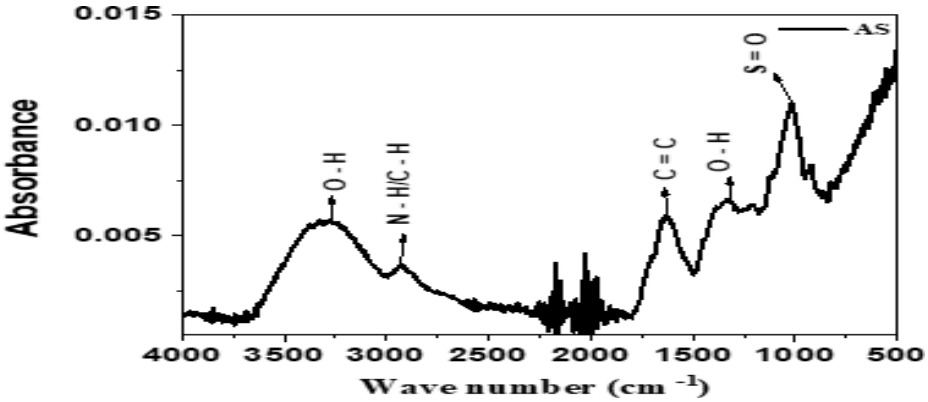


Figure 4: FT-IR Spectra of *Allium Sativum* (AS)

Table 1: Vibrational frequencies and wave number of *Allium Sativum*

Wave number (cm^{-1})		**Vibrational frequency**
3324.03	O-H stretching	alcohol (strong)
2931.11	C-H/N-H stretching	alkane (medium)
1634.40	C=C stretching	alkane (medium)
1330.69	O-H bending	phenol (medium)
1017.83	S=O stretching	sulfoxide (strong)

Table 2: IC50 of DPPH

Samples	**IC50**
Allium sativum AgNPS	396.7
Allium sativum methanol	11120000

IC50 = Inhibitory concentration at 50

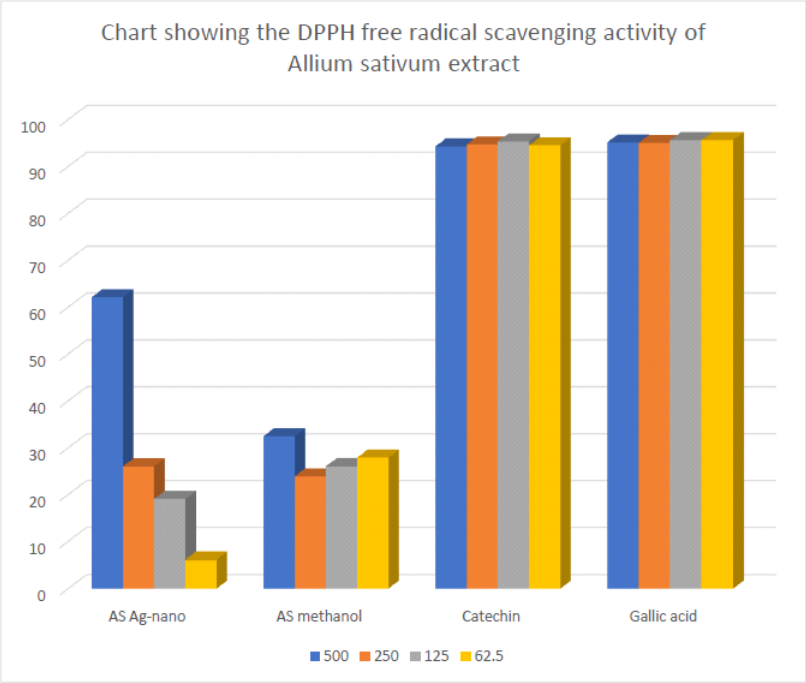


Figure 5: DPPH of AS Sample

Keywords: AS Ag-nano = *Allium sativum* AgNPS, AS methanol = *Allium sativum* methanol

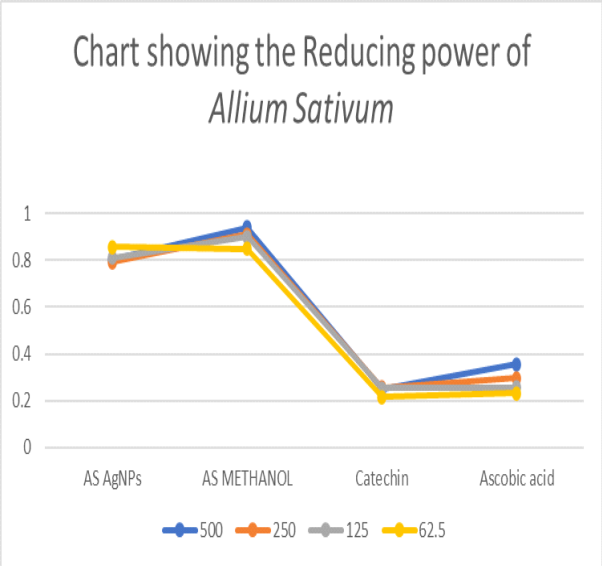


Figure 6: Reducing Power of As

Keywords: AS Ag-nano = *Allium sativum* AgNPS, AS methanol = *Allium sativum* methanol

The chromatogram below shows the peak numbers and the compounds with each wave length.

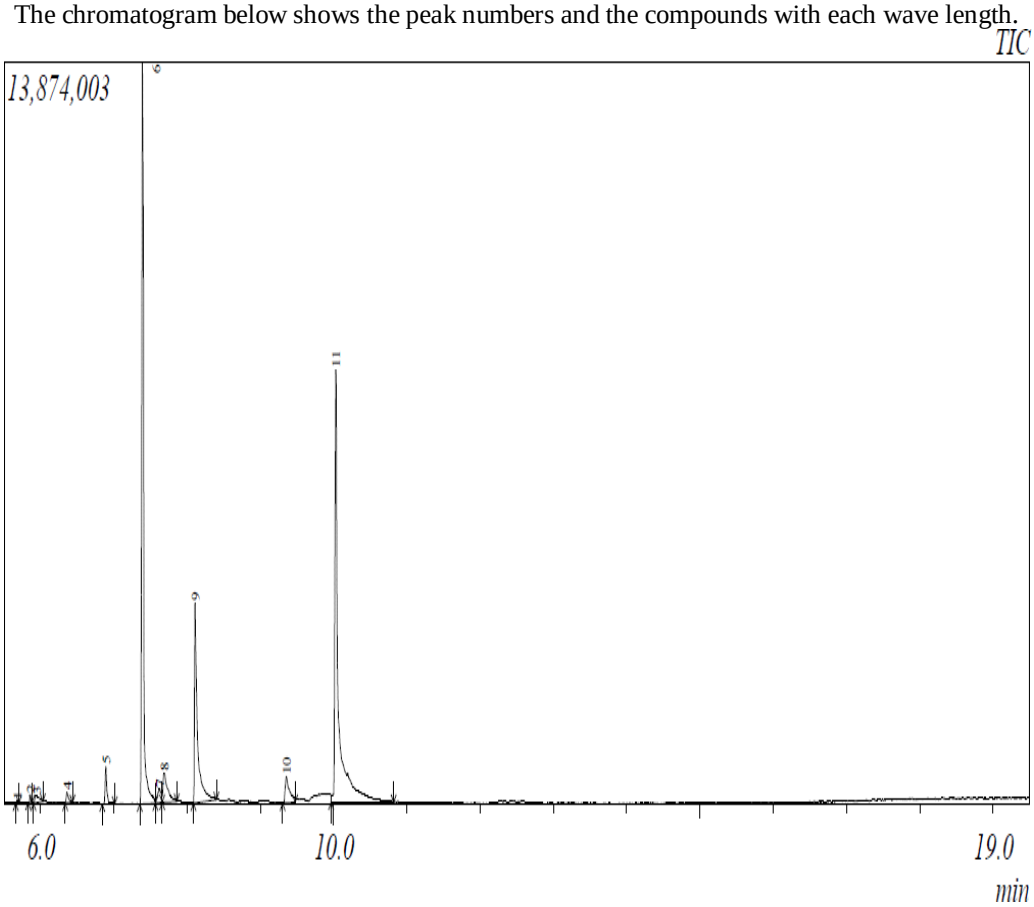
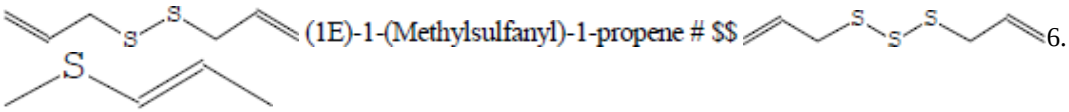


Figure 7: Chromatogram of *Allium Sativum*



These are compounds with the highest percent compositions

Table 3: Bioactive Compounds of *Allium Sativum*

Peak No	Ret Time	% Composition	Name
1	5.678	0.06	Ethanol, 2,2'-(nitrosoimino) bis-
2	5.859	0.25	Camphene
3	5.944	0.78	Dimethyl trisulfide
4	6.361	0.66	beta.-Myrcene
5	6.891	1.83	D limonene
6	7.392	34.48	Diallyl disulphide
7	7.621	1.35	Diallyl tetrasulphide
8	7.686	3.40	Tetrasulfide, di-2-propenyl
9	8.110	15.30	1 propene, 1-(methylthio)-, (E)
10	9.351	3.07	Verbenol
11	10.028	38.83	Trisulfide, di-2-propenyl

III. Discussion

Silver nitrate used for the synthesis of AgNPS (Hyllested *et al.*, 2015) which also play a role in the colour change, that changes from light yellow to dark brown. Colour change is an important factor for the synthesis of AgNPs. AgNPs appear brown in aqueous medium as a result of surface Plasmon vibrations Banerjee *et al.* (2014).

SEM analysis (figure 3a) of the AgNPs reveals the size, shape, morphology and organization because AgNPS have ability to agglomerate as a result of high surface tension and high surface energy in the extreme fine particles of AgNPS, Theivasanthi and Alagar, 2012. The EDX (3b) is used to confirm the presence, formation of the AgNPS and measures the distribution of X-ray signal generated by an electron beam on a specimen which was confirmed by the AgNPS synthesis of the extract carried out (Song and Kim, 2009). Ahmad *et al*, (2010), stated that the weak peaks from the EDX was a result of biomolecules bounded to the surface. Finally, the EDX of each sample shows the presence of some compounds that can be used in different applications. Those compounds are: Ag L (Silver iodide), Ci K (Potassium chloride), C K (Cyanogen chloride), P K (Phenol), S K (Potassium).

The FT-IR (fig 4), is used to reveal the AgNPS, capping and reducing the particular biomolecule from the functional group for identification, also play a role as identification and characterization of functional groups. (Liu *et al* 2007; Khanna and Nair, 2009; Sasidharen *et al.*, 2011) reported similar observations fromthe FT-IR. The table 1, shows the compounds, some of the functional group, belonging to hydrocarbons, esters, alcohols, acids which are mostly (monoterpenes and sesquiterpenes of bioactive compounds seen in 90% of the GC-MS essential oil). The esters are very important in aroma of food because the low carbon atoms of ester are highly volatile at ambient temperatures and the perception thresholds are much higher (Nogueira *et al*., 2005). Plant flavours and are used in food flavouring industries. Example, in Korean, organic acids have been used as major aroma compounds in barley bran (Steinhaus and Schieberle, 2007).The alcohol has been shown to have antibacterial and promote shelf-life in food (Onyenekwe *et al.*, 2012).Bioactive, like coenzyme, vitamins, iron, calcium, curcumin, etc., have been widely tested in nano delivery systems (He and Hwang, 2016). Different nano-delivery vehicles have been developed such as association colloids, lipid-based nanoencapsulations/nanocarriers, nano-emulsions, biopolymeric nanoparticles, nanolaminates, nanofibers, etc. These nano-delivery systems can increase the bioavailability of bioactive by different pathways. Nanoencapsulation can enhance bioavailability of bioactive compounds after oral administration through targeted delivery systems. Such nanoencapsulation enables to control the release of flavors at the desired time and also to protect the degradation of these flavours during processing and storage(Yu *et al.*, 2018).

Nowadays, people are requiring more nutritional supplements because many nutrients in food are being destroyed in the digestive tract. Each part presents a completely different environment, from oral cavity to the colon. In other words, there are a number of factors which decide the absorption of food in the body for infants, children, adults, old people, and those who are suffering from any type of gastrointestinal diseases. A nutrition

delivery system is a system or nanocarrier that delivers nutrition to specific places (Maestrelli *et al.,* 2006). Although a delivery system has numerous functions, one of them is to transport a functional ingredient to its desired site. Just like taste, texture, and shelf-life, major functions of a delivery system fora food product are that it should protect an ingredient from chemical or biological degradation, such as oxidation, and controlling the rate of release of functional ingredient under specific environmental conditions. Nano dispersions and nano capsules are ideal mechanisms for delivery of functional ingredientsbecause they can effectively perform all these tasks.

One of important part of the food industry is extracting nutrition from raw materials. Conventional methodsfor food processing are being replaced by newer techniques like nanotechnology, which will play a major role here. These techniques may improve food processing yields and decrease waste or spoilage of nutrition. Nutrition delivery systems must be prepared with biodegradable materials to prevent adverse effects on health of consumers.

Antioxidants control oxidative reactions by inhibiting, delaying, or hindering the oxidation of the biomolecules (Kumar *et al.*, 2011). Non enzymatic antioxidants can also neutralize radicals for example water soluble substances such as Vitamin C, glutathione, or fat-soluble substances such as Vitamin E, β- carotene (Trombino *et al.,* 2004). In recent years there has been an increased in the search for effective, non-toxic, natural compounds with antioxidative activity. Some nanomaterials have been seen to exhibit strong antioxidant properties. In this study, antioxidant activity of the synthesized AgNPs and corresponding methanol extract of the plants were studied by analysing antioxidant capacities which are indicative of the antioxidant potential of the synthesized AgNPs.

Vivekanandhan *et al.*, (2012) also reported DPPH as a stable organic free radical that has been used for investigating the free radical activities and thus antioxidant activity of various natural products (Rusherder *et al.,* 2012). The DPPH was a model of lipophilic radical. A chain reaction in lipophilic radicals which was initiated by lipid auto-oxidation. Being a stable free radical, DPPH is regularly used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm and its absorbance decreases upon reduction with an antioxidant (Lateef *et al.,* 2015).

The DPPH in figure (5) shows that the AgNPS of AS at 500 has a better activity than that of AS mathanolat 500, while the conc of 250, 125 and 62.5 were higher than that of AgNPS of AS respectively. The control (Catechin and Galic acid) has a higher overall better DPPH activity than the AS AgNPS and methanol. It is important to note that there is a concentration dependency in AS AgNPS.

The Reducing Power of a compound is related to its electron transfer ability and therefore may serve as a significant indicator of its potential antioxidant activity (Gülçin *et al.,* 2003). The reductive capabilities of the biosynthesized AgNPs and methanolic extracts are shown in figure (6) above. The reducing power of the samples increased with increasing number of concentrations. The reducing property of the extracts implies that it is capable of donating hydrogen atom in a dose dependent manner. The Reducing power of

A. Sativum of AgNPs and Methanol exhibited a better antioxidant activity than the Catechin and Ascorbicacid control from 500µg/ml to 62.5µg/ml respectively.

The identification of various bioactive compounds present in *A. Sativum* (table 3) oil were determined by GC-MS, full scan from the Chromatogram is on figure 5. The reference peak and retention time were noted.The oil yield was 0.3 ml, and the colour is transparent and appears light. The major bioactive compound analysed by the GC-MS were found to be 11 in number, some are: Diallyl disulphide (34%), Tetrasulfide,di-2-propenyl (3.40%), 1 propene, 1-(methylthio)-, (E) (15.30%), Verbenol (3.07%), Trisulfide, di-2- propenyl (38.83%), D limonene (1.83%). These values correspond to almost the values reported by Davut*et al* (2014) and Satyal *et al.* (2017).

The report by Davut *et al.* (2014) were Di-2-propenyl trisulfide (0.99%), Diallyl disulphide (41.87), 1propane (thiobis) 3,3 (2.11%), other compound like 1 propane, tetrasulfide, verberol etc maybe because the author didn't use essential oil in their analysis. Diallyl disulphide and trisulfide found in garlic bind to specific cysteine residues in B-tubulin to form S-allylmercaptocystein, because of this molecular basis of activity (Seki *et al.,* 2008) concluded that garlic has an anticancer property for those who consume them. Zhang *et al.* (2008), also reported that these compounds have anticancer activity.

A. Sativum has antioxidant properties because the Diallyl disulphide compound quenches hydroxyl radicalsgenerated by Tenton-type reactions (Rabinkov *et al.,* 1998). There is a high percentage composition of the sulphur compounds and which corresponds with Rabinkov *et al.,* 1998 that AS has abundant sulphur containing amino acids and other compounds that seem to initiate in increased activity in the immune system by stimulating immune function and making the killer cells more active. Therefore, *Allium sativum* is a healthy nutrient supplement that help to build and support the immune system, especially those who experience unhealthy lifestyle (chemical pollution, injury, mental tension, smoke, inadequate nutrition) Suliman *et al.,* 2006.

Studies also reveal that the compounds found in *Allium sativum* supports (antiviral activity) with bone marrow transplant and can reduce occurrence of common cold. The presence of S-allyl and Cysteine in garlic corresponds to Srivastava *et al.* (1995) that the presence of those compounds shows that *Allium sativum* has

anticarcinogenic effects and provides protection against liver damage.

IV. Conclusion

The essential oil of *Allium sativum* show good number of bioactive compounds. The Bioactive compounds has a lot of health benefits. Ci methanol extract and the synthesized Ci AgNPs could be used in nanomedicine and in nutraceuticals.

Furthermore, the AgNPs which is a new novelty, shows that the synthesized extract can be used for making not only food product but food film packaging material that will prevent peculation of chemical from the packaged material into the food, thereby leaching chemical/toxin substances into the food. If this is done, nutritional content of the food will be retained, shelf-life will be enhanced, and the food will be safe for human consumption.

Conflict of interest: The authors declare there was no conflicts of interests.

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