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# Physicochemical and Antimicrobial Activity of Phytochemicals from pods Extracts of Aframomum melegueta.

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**ABSTRACT**

*Aframomumsceptrum* is a tropical herbaceous perennial plant of genus *Aframomum* belonging to the family zinberaceae. The phytochemical analysis revealed the presence of flavonoids, phenolics, antocyannins and alkaloids as the active chemical constituents. The physicochemical assessment showed the pod $p^H$ was 6.7, moisture content and ash content were 71% and 14% respectively. The antimicrobial sensitivity profile revealed that pefloxacin was most effective against *Staphiloccous aureus* while chloramphenicol was effective on *E. coli*. The antifungal sensitivity profile showed that ketonidiazone was most effective against *Aspergilliusniga* while micotine was effective on *Candidalalbican.*Ethyalcetate extracts had minimum inhibitory concentration (MIC) at 250mg/ml and minimum bacterisidal concentration (MBC) at 100 mg/ml. Methanolic extracts has MIC of 150mg/ml against *E. coli* and minimum bactericidal of 50mg/ml respectively. Ethylactate extracts showed MIC and MBC of 100mg/ml and 10mg/ml against *S.aureus*. This study, therefore, showed that the phytochemical constituents were responsible for the antibacterial and antifungal activities recorded.

*Keywords*: Phytochemical, Physicochemical Antimicrobial

## Introduction

Most natural products of plants origin act as antibacterial and antifungal agents and have the potentials to control bacterial and fungal diseases of crops. Extracts and plant -derived chemicals of fruits, pods, leaves and root of some tropical Africa spicy plants have been variously used as protectants of poor - harvest and field pests for decades

Traditionally, *Aframomummelegueta* has been use for treatment of various ailments such as cough, diabetes mellitus, sore throat, stomach ache, erectile dysfunction and skin infections. In spite of these ethnomedical uses, there is dearth of documented information on the effectiveness of pod extracts of *A. melegueta*for the treatment of diseases.

The use of synthetic bactericidal and fungicides has been restricted through importation. Some industrially manufactured bactericide and fungicides have been band from the international market because of the health hazard and the danger them post to the environment. Many of them are toxic to both target and non-target organisms even to human. The need therefore, to look for antimicrobial substances that are from natural source which are biodegradable and environmental friendly and that can be used as alternatives to synthetic antimicrobial cannot be jettisoned.

## Materials and Methods

**Sample collection:** Dry fruits of *Aframomummelegueta* were purchased from Oja Oba market, Ilorin, Kwara State, Nigeria. The fruits were identified and authenticated in the Department of Pure and Applied biology, Ladoke Akintola Univerity of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria. The voucher specimen number was deposited in the Herbarium of Ladoke Akintola Univerity of Technology (LAUTECH), Ogbomoso, Nigeria.

**Preparation of Pod extracts:** The pod of the *Aframomummelegueta* were removed from the seeds and then kept in labelled polythene bag. They were later ground into a powdery form using Thomas - Wiley attrition machine. The powdered sample of *A. melegueta* was then stored in air tight aluminum foil inside the refrigerator for analysis.

**Extraction of Pod of *A. melegueta:*** The pod of the plant *A. melegueta* was left to dry at normal room temperature for weeks to remove moisture from the pulp. The dried pod was then grinded homogeneously to powder using sterilized blender and later soaked into two different solvents. The solvents used in this study are ethyl acetate and methanol. 100grams of the blended sample will be mixed with 500mls of ethyl acetate and methanol respectively at ambient temperature and shaken at 150rpm for 48hours, after which the extracts will be filtered with what man filter paper no l. The mixtures will further be filtered using 0.45µ Millipore filter. The filtrates will then be evaporated to semi solid mass using vacuum rotary evaporator to give a dark brown mass and stored at $4^0$c in a dark bottle prior to use (Kozya*et al.*2015).

**Phytochemical screening of *Aframomummelegueta*:** The Pod extracts of *Aframomummelegueta* were screened for the presence of alkaloids, flavonoids, phenols, saponins and steroids. One percent (%) of each extract was prepared as stock solution by weighing 1gm of each and dissolved in 100 ml of distilled water (Bergeon*et al.* 2015; Beejmohun*et al.,* 2017; ).

**Test for phenols:** Two drops of 5% $FeCl_3$ was added to 1 ml of the extract solution in a test tube, and a greenish precipitate confirmed a positive test for phenol.

**Test for flavonoids:** One ml of 10 % NaOH was added to 3 ml of the extract solution, development of yellow coloration was taken as a positive confirmation for presence of flavonoids.

**Test for alkaloids:** One ml of 1 % HCl was added to 3 ml of the extract and heated for 20 min followed by addition of 2drops of Mayer's reagent, formation of a creamy precipitate indicated the presence of alkaloids.

**Test for saponins:** Two ml of the extract solution in a test tube was shaken vigorously and observation made as an indication for the presence of saponins.

**Test for steroids** Two ml of chloroform was added to the extract solution and the mixture filtered. The filtrate was treated with three drops of acetic anhydride. This was bolied and allowed to cool. Five drops of concentrated $H_2SO_4$ was added. Development of a brown ring colour indicated the presence of steroids.

**Reconstitution of the pod extract of *A. melegueta:*** The dried extracts will later be reconstituted with their respective extractants (ethylacetate and methanol) to give concentrations of 10. 50, 100. 150, 200, 250 all in mg/ml for the antimicrobial and antifungal activity evaluation.

**Preparation of Potato Dextrose Agar (PDA):** 9.5g of Potato Dextrose Agar was dissolved in sterile conical task which contained 250ml of distilled water. The suspension was plugged with cotton wool wrapped with aluminum foil which was heated to boiling on a Bunsen burner. It was autoclaved at $121^0$C for 15 minutes. After autoclaving, the media was allowed to cool to a temperature of about $45^0$ C and 5ml of 1% streptomycin was introduced to it before pouring into petri-dishes.

**Preparation of Nutrient Agar (NA):** 7g of Nutrient Agar was dissolved in sterile conical flask which contained 250ml of distilled water. The suspension was plugged with cotton wool wrapped with aluminum foil which was heated to boiling on a Bunsen burner t was autoclaved at 2TCfor 15 minutes. After autoclaving, the media was allowed to cool to a temperature of about $45^0$ C before pouring.

**Pure culture of selected Organisms:** Each of the selected organisms were resub cultured to obtain pure culture and the pure cultures were then inoculated into Nutrient Agar(NA) and Potato Dextrose Agarm (PDA) respectively on a slant of McCartney bottles for stocking The stock was used for the analysis.

**Microscopic Examination of Pathogens:**The pure culture of the selected microorganisms were 0btained from Microbiology department, University of Ilorin, Kwara State, Nigeria was used for the purpose of this project works. Each of the selected microorganisms was subjected to colony and microscopic examination during which their structural features were observed under the microscope. The characteristics observed were matched against those available in manuals of Barnett and Hunters (1985). They were then ascertained.

**Antimicrobial and Antifungal Activity of the pod extracts:** This was done as described by Adomi (2008). Each concentrated extract will be dissolved in 5ml volume of *ethylacetate* and methanol respectively to give the desired concentration of extract in milligram. 1ml of Mcfarland standard of 18hour culture of each of the bacterial isolates (*staphylococcus amrous*and *Escherichia coli*) will be mixed with 20ml of nutrient agar in petri-dishes. Also, 1ml of Mcfarland standard of 18hours culture of fungal isolates (*Aspergellusniger* and *candillaalbcans*) will be mixed with 20mls of potato Dextrose Agar PDA) in petri-dishes. Seven wells of 5mm in diameter will be bored in the agar medium using sterile cork borer before been filled with 1ml of the plant extract and extractants (control) and will be allowed to diffuse for 2hours (Akpulu,2004, Akroun, *et al,*. 2012), The Nutrient agar plates will be incubated for 18-24hr at $37^0$c while the potato dextrose agar plates will be incubated at ambient temperature for 72hours. The diameter of any resulting zone of inhibition will be measured.

**Determination of Minimum Inhibition Concentration (MIC) of the Pods Extracts:** The minimum inhibitory concentration of the extract was determined using the method of Julius *et al*. (2017).

The dilutions of the plant extracts were prepared and 1ml of each of the different concentrations of the solution was added to 9mls of sterile molten nutrient agar and potato dextrose agar respectively at $40^0$c to give concentrations of 10, 50, 100, 150, 200, 250mg/ ml The medium was then poured into sterile petri-dishes and allowed to dry before streaking with 18hours old selected isolates. The petri-dishes were incubated at $37^0$c for 24hours for bacteria growth while that of potato dextrose agar plates will be incubated at ambient temperature and fungi growth will be examined after 72hours (Kumarasamy*et al.* 2013,) All the plates will be examined for presence or absence of growth. The MIC was taken as the least concentration that prevent bacterial and fungal growth respectively,

**Determination of Minimum Bactericidal Concentration (MBC) of Pods Extract:** The concentration of the extracts used for the MIC which do not permit any visible microbial growth will be inoculated on petri-dishes containing the growth medium and incubated for 24hours. The least concentration which show no growth after incubation will be taken as the minimum bactericidal concentration (akoun*et al.*2012).

**$P^H$ Determination of the Pod:** Five gram of the pod sample of *A. melegueta* was dispersed into 50mls of distilled water in a conical flask. The suspension was mixed thoroughly and allowed to settle for 10 minutes. The $P^H$ was then taken. The procedure was done in duplicate for the sample and the mean result was recorded.

**Determination of Moisture Content of the Pod:** A clean crucible was dried in an oven for 30minutes and cooled in a desiccator. The cooled crucible was weighed as $W_1$. Then 5g of pod sample of *A. melegueta* was introduced into the dried crucible and weighed as $W_2$ before drying. This was then put in an oven set at $80^0$c for an interval of 2hours and cooled in a desiccator and reweighed as $W_3$, this was carried out until constant. The moisture content was calculated thus,

Weight of oven dry crucible =$W_1$

weight was obtained.

Weight of oven dry crucible + sample =$W_2$

Weight of oven dry + dried sample =$W_3$

Total loss in weight =$W_3$-$W_2$

Weight of sample =$W_2$-$W_1$

%Moisture content = $w2 - w1$

**Determination of Ash Content of Pod:** A clean oven dried crucible was weighed as $W_1$. 5g of the pod sample of the pod sample of *A. melegueta* was added into the clean dried crucible and weighed as $W_2$. The crucible and its content was then transferred into the muffle furnace set at 600'c tor about 6hours, the colour change shows that it was fully ashed. The crucible and its content was removed from the furnace and placed in the desiccator to cool after cooling it was then reweighed as $W_3$.

The ash content of sample was calculated as thus;

Weight of oven dried crucible =$W_1$

Weight of oven dried crucible + sample =$W_2$

Weight of dried crucible + sample (after ashing) =$W_3$

Weight of sample- ($W_2$-$W_1$) g

Weight of Ash = ($W_3$-W1) g

%Ash content = $w2 - w1$

**Table 1:** Phytochemical screening of Pod extracts of *Aframomummelegueta*

| Extracts | Flavonoids | Alkaloid | Phenolics | Saponins | Steroids |
|---|---|---|---|---|---|
| Ethyl acetate | + | + | + | + | _ |
| Methanolic | + | + | | + | _ |

Keys : (+) means present; (-)means not detected

**Table 2:** Physicochemical characteristics of pod powder of *Aframomummelegueta*

| Sample | PH | Moisture content (%) | As h contents (%) |
|---|---|---|---|
| Pod powder | 6.2 | 71 | 14 |

**Table 3:** Antibiotics Sensitivity profile of selected bacterial

| Bacterial | Zones of inhibition in mm | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PEF | PFX | S | SXT | CH | SP | CPX | AM | AU | CN |
| *Staphylococcus aureus* | 22 | 17 | - | - | - | 15 | 18 | 12 | 18 | - |
| *Escherichia coli* | 14 | - | 10 | 20 | 25 | 20 | 18 | - | 14 | - |

Key: PEF- Pefloxacin, OFX-Oflxacin, SXT- Safloxacin, CH-Chloraphenicol, S-Septrin, SP- Spectinomycin, CPX- Ciprofloxacin, AM- Amoxixillin, AU- Augumentin, CN- Cocistin.

**Table 4:** Antifungal Sensitivity profile of selected fungal

| Fungi | Zones of inhibition in mm | | | | | |
|---|---|---|---|---|---|---|
| | FUS | FLU | KET | CLO | DON | MYC |
| *Aspergillus niger* | 16 | 10 | 18 | 12 | 10 | - |
| *Candida Albican* | 12 | - | 10 | - | 12 | 15 |

Key: FUS-Fusclin, FLU-Fluconidazole, KET-Ketronidazole, CLO-Cloxtrimazole, DON-Donystatine, MYC-Mycoten.

**Table 5:** Sensitivity test exhibited by Pod of *Aframomummelegueta* extract on *Escherichia coli*

| Concentration (mg/ml) | Organism | Zones measurement in (mm)-Extractions | |
|---|---|---|---|
| | | Ethyl acetate | Methanol |
| 10 | *Escherichia coli* | 15 | 11 |
| 50 | *Escherichia coli* | 18 | No inhibition |
| 100 | *Escherichia coli* | 20 | No inhibition |
| 150 | *Escherichia coli* | 21 | No inhibition |
| 200 | *Escherichia coli* | 25 | No inhibition |
| 250 | *Escherichia coli* | 23 | 17 |
| Control | *Escherichia coli* | 3 | 5 |

**Table 6:** Sensitivity test exhibited by Pod of *Aframomummelegueta* extract on *Staphylococcus aureus*

| Concentration (mg/ml) | Organism | Zones measurement in (mm)-Extractions | |
|---|---|---|---|
| | | Ethyl acetate | Methanol |
| 10 | *Staphylococcus aureus* | 10 | 18 |
| 50 | *Staphylococcus aureus* | 15 | 16 |
| 100 | *Staphylococcus aureus* | 5 | 21 |
| 150 | *Staphylococcus aureus* | 17 | 13 |
| 200 | *Staphylococcus aureus* | 23 | 10 |
| 250 | *Staphylococcus aureus* | 7 | 5 |
| Control | *Staphylococcus aureus* | 5 | 1 |

**Table 7:** Sensitivity test exhibited by Pod of *Aframomummelegueta* extract on *Apsergillusniger*

| Concentration (mg/ml) | Organism | Zones measurement in (mm)-Extractions | |
|---|---|---|---|
| | | Ethyl acetate | Methanol |

| | | | |
|---|---|---|---|
| 10 | *Apsergillusniger* | 20 | 11 |
| 50 | *Apsergillusniger* | 10 | 15 |
| 100 | *Apsergillusniger* | 20 | 10 |
| 150 | *Apsergillusniger* | 8 | 12 |
| 200 | *Apsergillusniger* | 12 | 7 |
| 250 | *Apsergillusniger* | 10 | 8 |
| Control | *Apsergillusniger* | No inhibition | No inhibition |

**Table 8:** Sensitivity test exhibited by Pod of *Aframomummelegueta* extract on *Candida albican*

| Concentration (mg/ml) | Organism | Zones measurement in (mm)-Extractions | |
|---|---|---|---|
| | | Ethyl acetate | Methanol |
| 10 | *Candida albican* | 20 | 18 |
| 50 | *Candida albican* | 20 | 16 |
| 100 | *Candida albican* | 17 | 20 |
| 150 | *Candida albican* | 22 | 18 |
| 200 | *Candida albican* | 18 | 20 |
| 250 | *Candida albican* | 24 | 18 |
| Control | *Candida albican* | No inhibition | No inhibition |

**Table 9:** Showing MIC of the Ethyl acetate extract of the Pod of *Aframomummeleguetaon E.coli and S.aureus*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Esxherichia coli* | + | + | + | + | + | (*) - |
| *Staphylococcus aureus* | + | + | (*) - | - | - | - |

Key: (*) = MIC concentration, + = Growth, - = No growth

**Table 10:** Showing MIC of the Methanol extract of the Pod of *Aframomummelegueta*on *E.coli and S. aureus*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Esxherichia coli* | + | + | + | (*) - | - | + |
| *Staphylococcus aureus* | (*) - | + | + | + | - | - |

Key: (*) = MIC concentration, + = Growth, - = No growth

**Table 11:** Showing MIC of the Ethyl acetate extract of the Pod of *Aframomummelegueta*on *A. niger and C. albicans*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Aspergillus niger* | + | (*) - | - | - | - | - |
| *Candida albican* | (*) - | - | - | + | - | - |

Key: (*) = MIC concentration, + = Growth, - = No growth

**Table 12:** Showing MIC of the Methanol extract of the Pod of *Aframomummelegueta*on *A. niger and C. albicans*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Aspergillus niger* | (*) - | - | - | - | - | - |
| *Candida albican* | + | + | + | (*) - | - | + |

Key: (*) = MIC concentration, + = Growth, - = No growth

**Table 14:** Showing MBC of the Ethyl acetate extract of the Pod of *Aframomummelegueta*on *E.coli and S. aureus*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Esxherichia coli* | + | + | (*) - | + | + | + |
| *Staphylococcus aureus* | (*) - | + | - | - | - | - |

Key: (*) = MIC concentration, + = Growth, - = No growth

**Table 15:** Showing MBC of the Methanol extract of the Pod of *Aframomummelegueta*on *E.coli and S.aureus*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Esxherichia coli* | + | (*) - | + | + | + | + |
| *Staphylococcus aureus* | + | (*) - | + | + | - | - |

Key: (*) = M IC concentration, + = Growth, - = No growth

**Table 16:** Showing MBC of the Ethyl acetate extract of the Pod of *Aframomummelegueta*on *A. niger and C. albicans*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Aspergillus niger* | + | (*) - | - | - | - | - |
| *Candida albican* | (*) - | - | - | - | - | - |

Key: (*) = MIC concentration, + = Growth, - = No growth

**Table 17:** Showing MBC of the Methanol extract of the Pod of *Aframomummelegueta*on *A. niger and C. albicans*

| Test Organism | Concentrations in (mg/ml) of extracts | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 250 |
| *Aspergillus niger* | (*) - | - | - | - | - | - |
| *Candida albican* | (*) - | - | - | - | - | - |
| *Aspergillus flavus* | (*) - | - | - | - | - | - |
| *Aspergillus fumigatus* | (*) - | - | - | - | - | - |

Key: (*) = MIC concentration, + = Growth, - = No growth

***Results***

The flavonoids, alkaloids, phenolics and antocyannins were present in the extracts tested as shown in table 1. Physicochemical assessment showed that pod powder pH was 6.2, moisture content was 71% and ash content was 14%. (Table 2).

The antimicrobial sensitivity profile was determined using disk method and zones of inhibition were measured, standard antibiotics were use as control. It was review that pefloxacin was most effective against staphylococcus aureus while chloramphenicol was effective on *E. coli*. The antifungal sensitivity profile showed that ketonidiazone was most effective against *aspergilliusniga* while micotine was effective on *Candidalalbican.*Ethyalcetate

extracts had minimum inhibitory concentration (MIC) at 250mg/ml and minimum bacterisidal concentration (MBC) at 100 mg/ml. Methanolic extracts has MIC of 150mg/ml against E. coli and minimum bactericidal of 50mg/ml respectively. Ethylactate extracts showed MIC and MBC of 100mg/ml and 10mg/ml against *S. aureus*

***Discussion***

The rising prevalence of antibiotics resistant pathogenic microorganisms in the last decades raises the demand for finding new alternative antimicrobial agents. Therefore, the current study evaluated the antimicrobial potential of Pod extracts of *A.melegueta* which have potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents. Pod extracts of *Aframomummelegueta*have been screened for their antimicrobial activities on resistant strains of bacteria and fungi. The extracts showed antimicrobial activity in a dose dependent manner against the tested organisms. The obtained results show that ethyl acetate and methanol extracts had wide antibacterial and antifungal spectrum against test bacterial and fungal strains. Therefore, extracts as a result of their inhibitory effects against gram-negative bacteria can be developed for the use.

### *3.3Preparation of Sample*

Dried *Cannabis sativa* leaves were first milled and then screened using a 20-mesh sieve. To obtain the leaf extract of *Cannabissativa*, 1g of the screened *Cannabis sativa* leafpowder was weighed and dispersed in 250mL distilled water. The solution was then heated in water bath at $60^0$C for an hour. The mixture was filtered using Whatman No. 1 filter paper to remove the residual insoluble biomass, and the resulting filtrate was then centrifuged at 4000 rpm for 15 minutes.

### *3.4Preparation of Gold Chloride ($HAUCL_4$) Solution*

The gold chloride solution was prepared by dissolving 0.34g of $HAuCL_4$ into 1000ml of distilled water at ambient temperature and was stored into amber bottle and kept in a dark cupboard.

### *3.5Biosynthesis of Gold Nanoparticles*

2 ml of the leaf extract was drawn using a sterile hypodermal syringe into three different slant bottles and were labelled accordingly and 20ml of gold chloride (1 $mMAuCl_3$) aqueous solution was added into each of the bottles containing 2 ml of the leaf extract in ratio 1:10 and was labelled. The solution containing the aqueous salt solution and the leaf extract were then photo-activated by exposure to direct sunlight as it enhances the formation of the gold nanoparticles. As the experimental control, a solution of the aqueous solution alone was also maintained. The formation of AuNPs was visually observed by the colour change to violet.

### *3.6Physicochemical characterization of Gold Nanoparticles (AuNps)*

#### *3.6.1UV-Vis spectroscopy analysis*

Ultraviolet visible spectroscopy is a widely used technique to monitor the reduction of gold metal ions into gold nanoparticles; In this case, the reduction was confirmed by measuring the absorbance spectra using UV–vis spectrophotometer (BIOBASE-UV1800PC, LAUTECH Central Research Laboratory) to scan the reaction mixture in the range of 190.0-800.0nm. A quartz cuvette of 1cm path length was used to hold the sample and distilled water was used to as reference of the absorption spectra. UV–vis spectrophotometer uses light in the visible and adjacent near-UV and near-infrared (NIR) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved.

#### *3.6.2Fourier Transform Infra-red (FTIR) spectroscopy*

Functional groups in plant leaf extract and bio-fabricated AuNps was determined using FTIR spectroscopy because it is a highly diverse molecular spectroscopy technique and chemical analysis method. FTIR is frequently used for polymer testing and pharmaceutical analysis. Fourier transform infrared (FTIR) spectroscopic analysis was carried out using an IRAffinity-IS Spectrophotometer (LAUTECH, Central Research Laboratory) on the powdered form of gold nanoparticles sample according to the method of (Bhat et al., 2016). 5ml of gold nanoparticles solution was poured into a glass petri dish and was transferred into an oven at a temperature of $40^0$C for 4hours in other to change from the liquid to solid state. The solid residue obtained was cooled down at room temperature and the powder obtained was used for FTIR measurements using KBr pellets.

### *3.7Antifungal activities of synthesized AuNps of C. sativa*

The antifungal activities of the gold nanoparticles were evaluated using Mycelial Inhibition Method (Khatami et al., 2015; lateef et al., 2016) by incorporating graded concentrations (150 µl /ml) of the synthesized gold nanoparticles into potato dextrose agar plates, which were then inoculated with 6mm fungal plug of 48-hrs old cultures of *Aspergillus niger, Aspergillus flavus*, *Aspergillus fumigatus, Fusarium solani* and *Candida albican*. In the control experiments, fungal plugs were inoculated on PDA plates without the incorporation of the gold nanoparticles. All the plates were incubated

at 28 ± 2 C for 72 hrs. The diameters of fungal growths in all the plates were measured and used to determine the percentage growth inhibitions as follows:

$$\frac{D_{control} - D_{test}}{D_{control}} \times 100\ \%$$

where D is the diameter of fungal growth on the PDA plates.

### *3.8Anticoagulant and thrombolytic activities of synthesized AuNps of C. sativa*

The anticoagulant activities of the gold nanoparticles were investigated as earlier described by Azeez et al. (2017). Exactly 150 µl of the gold nanoparticles prepared as 100 µg/ml were added to 0.5 ml of blood freely provided by a healthy donor, while blood collected into EDTA and blood collected in clean Eppendorf tubes served as positive and negative control respectively. In addition, blood samples were treated with the 150 µl *Cannabis sativa* extract and aqueous gold chloride solution. All the set up were held at room temperature (30 ± 2 °C) for 30 min, and thereafter the tubes were inverted for anticoagulation examination. Subsequently, smears prepared from the samples were examined under Olympus microscope to observe the morphology of red blood cells (Azeez et al., 2017).

The thrombolytic activity was determined using the methods of Devi et al. (2016). In this case, thrombolytic activity was quantified (Lateef et al*.,* 2017). Eppendorf tubes containing 0.5 ml of blood were held at 37 °C for 30 min to allow the blood to clot. The weight of clean tube (W1) was subtracted from the weight of tube and blood clot (W2) to obtain the weight of blood clot (W3). Thereafter, 100 µl of the varied concentrations of the gold nanoparticles, and *Cannabis sativa* extract were added to each tube containing the blood clot. These were incubated at 37 °C for 30 min, and then inverted to check for clot lysis. The lysed clot was drained, and the weight of the tube with remaining clot was taken (W4) to obtain the weight of clot that was not lysed (W5). The percentage thrombolytic activity was obtained as:

The lysed blood clot was also examined microscopically as previously described to observe the morphology of red blood cells (Ojo et al., 2016; Lateef et al., 2017).

## 4. RESULT AND DISCUSSION

### 4.1 Biological synthesis of AuNps

*Cannabis sativa* leaf extract was added to the precursor and the photo-activation of the solution containing the aqueous salt solution and the leaf extract by exposure to direct sunlight within 30minutes led to a colour change of the reaction mixture from yellow to violet which stabilized in 1 hour of reaction. This was the first indication of nanoparticle synthesis and corresponds with the surface plasmon resonance (SPR) property of the formed AuNps (Fig.4.2). The color change owing to the formation of nanoparticles is due to the SPR excitation of the reduced metal particles, which was assisted by the bioreductant abilities of the phytochemicals present in the extract applied. The control (gold chloride solution without any sample) remained colourless throughout the photo-activation stage.

### *4.2 Physicochemical characterization of AuNps*

#### *4.2.1 UV–vis spectroscopy*

The UV-Vis absorbance spectra of the gold nanoparticles bio-fabricated by *Cannabis sativa* leaf extract are shown in (Figure 4.1). Metallic AuNps are known to exhibit absorbance range of 500-600nm attributed to surface plasmon resonance, the metallic AuNps synthesized exhibited absorbance peak at 580nm. This range of surface plasmon resonance has been suggested for AuNps according to literature.

#### FTIR Spectroscopy

The FTIR absorption spectra of the synthesized nanoparticles showed distinct and conspicuous bands at 3526, 3050, 2417, 1674, 1398 and 1078 for biosynthesized AuNPs (Figure 4.4). 3526 and 3050, corresponding to O-H band stretching or N-H stretch of amine, 2417 corresponding to C-H stretching, 1674 corresponding to C=C stretch of alkenes, 1398 corresponding to C-H bending, and 1078 corresponding to C-O stretching of ether. It was apparent from these bands that biomolecules rich in amines (N-H) and hydroxyl (O-H) from the leaf extract were accountable for the reduction in $Au^{+}$, as well as capping and stabilization of AuNPs.

### *4.3 Antifungal Activities*

The biosynthesized nanoparticles significantly inhibit the growth of *Candida albican, Aspergillus niger, Aspergillus flavus, Fusarium solani and Aspergillus Fumigatus* (Figure 4.5). The AuNPs synthesized showed inhibitions of 1.96%, 9.8%, 42.9%, 50%, and 1.25% against *Candida albican, Aspergillus niger, Aspergillus flavus, Fusarium solani and Aspergillus Fumigatus* respectively (Table 4.1)

### *4.4Anticoagulant activities*

Formation of clots was inhibited by the addition of the biosynthesized nanoparticles to fresh blood of healthy human donor as also observed in the positive control (blood with EDTA) whereas coagulation was noticed in the negative control (blood with only extract or salt solution alone). The gold prevents formation of coagulation (blood clot) when used as anticoagulant when compared to the positive control using EDTA. (Figure 4.6)

**Thrombolytic activities**

Furthermore, the nanoparticles displayed potent activities at causing dissolution of preformed blood clots within 5 min (Plate 4.4). Whereas, the control samples which included the preformed blood clot treated with C. sativa leaf extract, $AuCl_3$ solution revealed no thrombolytic activities in them (Table 4.2).

**plate 4.1**: Prepared leaf extract of *C. sativa*

**Plate 4.2:** Visible colour change of Biosynthesized AuNps using *Cannabis sativa* leaf extract.

**Figure 4.1:** UV- visible spectrum of the synthesized nanoparticles

**Fig 4.2:** UV- visible spectrum of the C. sativa leaf extract

**Fig 4.3:** UV- visible spectrum of the Gold chloride

**Figure 4.4:** FTIR spectrum of the synthesized nanoparticles

**Table 4.1:** Antifungal activities of the synthesized nanoparticles

| Organisms | Antifungal activities of synhesizedAuNps (%) |
|---|---|
| *F. solani* | 50 |
| *C. albican* | 1.96 |
| *A. niger* | 9.8 |
| *A. flavus* | 42.86 |
| *A. fummigatus* | 1.25 |

**Figure 4:** Antifungal activityof the synthesized nanoparticles

**Plate 4.3:** Antifungal activityof the synthesized nanoparticles

**Table 4.2:** Thrombolytic activity of AuNPs

| Stages | $W_3$ | $W_5$ | |
|---|---|---|---|
| Control | 0.4 | 0.4 | 0 |
| Clot + extract | 0.4 | 0.3 | 25 |
| Clot + AuNps (Conc. A) | 0.4 | o.3 | 25 |
| Clot + AuNps (Conc. B) | 0.4 | 0.6 | 50 |
| Clot + AuNps (Conc. C) | 0.4 | 0.5 | 25 |
| Clot + AuNps (Conc. D) | 0.4 | 0.4 | 0 |

Key:

$W_3$ – weight of blood clot

$W_5$–weight of blood that was not lysed

**Figure 4.6:** Thrombolytic activities of AuNPs

**Plate 4.4:** Thrombolytic Activity of the synthesized Nanoparticles

**Plate 4** Anticoagulant Activity of the synthesized Nanoparticles

## DISCUSSION

The biosynthesis of AuNPs was mediated by the extract of *C. sativa* within 15 minutes under exposure to direct sunlight. The violet colour of biosynthesized AuNPs has been extensively reported by (Lateef *et al.,* 2016). The colour change from light yellow to violet shows the formation of Au nanoparticles (Shah *et al.,*2011). (Malathi*et al.,*2014) and (Ramakrishna *et al.,*2016) reported variation in the colour of Au NPs such as ruby brown, red pinkish, yellowish and purple due to the composition of bioactive molecules responsible for the synthesis of the nanoparticles which lend credence to the coloration i.e., violet colouration of the one synthesized in this study.

Characterization was carried out to monitor the formation of the gold nanoparticles through UV-Visible spectroscopy analysis and Fourier Transformed Infrared Spectroscopy (FTIR). Ultraviolet visible spectroscopy is a widely used technique to monitor the reduction of gold metal ions into gold nanoparticles; In this case, the reduction was confirmed by measuring the absorbance spectra using UV–vis spectrophotometer (BIOBASE-UV1800PC, LAUTECH Central Research Laboratory) to scan the reaction mixture in the range of 190.0-800.0nm. A quartz cuvette of 1cm path length was used to hold the sample and distilled water was used to as reference of the absorption spectra. UV–vis spectrophotometer uses light in the visible and adjacent near-UV and near-infrared (NIR)ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. The result of UV-Visible provides an insight into the size, distribution, surface properties and optical properties of Nanoparticles; as the wavelength of peak absorption depends upon several factors such as particle size, di-electric constant of surrounding media and the inter-particle distance (Huang and Yang, 2004). The UV-visible spectrum of the synthesized AuNP displayed maximum absorbance at the wavelength of 580. Also, the values obtained for AuNPs is within the range (400-600nm) reported by (Link *et al.,*1999).

Functional groups in plant leaf extract and bio-fabricated AuNps was determined using FTIR spectroscopy because it is a highly diverse molecular spectroscopy technique and chemical analysis method. FTIR is frequently used for polymer testing and pharmaceutical analysis, the application of the technique is virtually limitless offering both qualitative and quantitative analysis of a wide range of organic and inorganic samples. FTIR is able to provide accuracy, reproducibility and also a favourable signal-to-noise ratio. By using FTIR spectroscopy, it become possible to detect small absorbance changes on the order of $10^{-3}$, which helps to perform spectroscopy, which one could distinguish the small absorption and of functionally active residue from the large background absorptions of the entire protein (Gerwert et al., 1999). FTIR spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles, which is more pronounced in academics and industrial research (Lin et al*.,* 2014)

Fourier transform infrared (FTIR) spectroscopic analysis was carried out on the powdered form of AuNps sample using an IR Affinity –IS Spectrometer according to the method of (Bhat*et al.,*2016). For the synthesized AuNps, the broad peaks are 3526, 3050, 2417, 1674, 1398 and 1078, 3526 and 3050, corresponding to O-H band stretching or N-H stretch of amine, 2417 corresponding to C-H stretching, 1674 corresponding to C=C stretch of alkenes, 1398 corresponding to C-H bending, and 1078 corresponding to C-O stretching of ether. It was apparent from these bands that biomolecules rich in amines (N-H) and hydroxyl (O-H) from the leaf extract were accountable for the reduction in $Au^{+}$, as well as capping and stabilization of AuNPs.

The synthesized gold nanoparticles showed anticoagulation properties as the formation of clot was inhibited by their addition to fresh blood. The results obtained are in conformity with anticoagulant potentials of AuNPs synthesized from diverse biomolecules as previously reported by (Azeez *et al.,*2017). The AuNPs showed thrombolytic activities of 25, 50, 25, 0 at concentration A, B, C and D respectively. Therefore, the thrombolytic activities of the synthesized gold nanoparticles showed fair thrombolytic properties. Furthermore, AuNPs prevented coagulation of blood and also dissolved blood clot indicating the biomedical potential of AuNPs in the management of blood coagulation disorders (Ojo*et al.,* 2016). Though blood clotting is required to prevent bleeding, but its dissolution is equally important in preventing thrombosis and maintenance of hemostasis, of which nanoparticles play important roles in rendering efficient lysis of blood clots (Ilinskaya*et al.,* 2013). The anticoagulant and thrombolytic potentials of metallic nanoparticles have been in literature and also recently reported by (Oladipo *et al.,* 2017).

## CONCLUSION

In this investigation, an eco-friendly and cost-effective method was utilized for synthesis of AuNps by *C. sativa* aqueous leaf extract. The adopted methodology allowed production of nanoparticles AuNPs, at 1:10 ratio of plant extract and 1mM gold chloride**.** Characterization of the synthesized nanoparticles was carried out by different techniques including Ultraviolet-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope and Energy Dispersive Microscope. FTIR revealed the presence of flavonoids, cannabinoids, terpenes and phenols on the nanoparticle surface, which could be responsible for reducing the salts to nanoparticles and further stabilizing them. The morphology, antifungal, and anticoagulant and thrombolytic activities of the synthesized AuNps were measured by various methods. Results of this study illustrated that *C. sativa* leaf aqueous extract with various groups of phytochemicalssuch as phenols and flavonoids had suitable property in green synthesis of AuNps and therefore established the relevance of the *C. sativa* in nano-biomedical applications, particularly in green synthesis of low cost, eco-friendly, safe, reliable and stable Au nanoparticles. The biosynthesized AuNPs showed significant antifungal property on *Aspergillus niger, Aspergillus flavus*, *Aspergillus fumigatus, Fusarium solani* and *Candida albican*. The nanomedical significance of the nanoparticles in the potential management of blood coagulation disorders was established as AuNPs efficiently dissolved blood clots and also functioned as excellent thrombolytic agent. Therefore, this study has comprehensively established the potential applications of gold nanoparticles synthesized from *C. sativa* leaf extract as antifungal, anticoagulant, and thrombolytic agents. Results of this study illustrated that *C. sativa* leaf aqueous extract with various groups of phytochemicals such as phenols and flavonoids had suitable property in green synthesis of AuNps. However, concentrated surveys are necessary in order to determination of other aspects of NPs green synthesis such as chemical composition properties of leaf extract and their mechanisms effect.

### *5.2 RECOMMENDATION*

As revealed in this study, Gold nanoparticles synthesized using *C. sativa* possess significant antifungal property and exhibited potent anticoagulation and thrombolytic characteristics. It is, therefore, highly recommended to consider incorporation of Gold nanoparticles in antifungal treatment as well as in the control of blood coagulation and in dissolution of blood clots, either as a pretreatment, stand-alone treatment or used alongside other methods. However, further studies that will explore other potential health benefits of the Gold nanoparticles should be delved into by researchers across the globe.

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