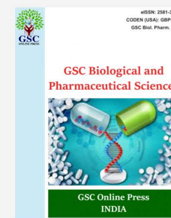
GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/




(RESEARCH ARTICLE)

# Comparative analysis of phytochemical screening and antimicrobial properties of green synthesized nanoparticle and *Hibiscus Sabdariffa* extract

Monica Mmachukwu Okorondu [1,*], Cosmos Onyekachi Ujowundu [1], Sylvester Ifunanya Okorondu [2] and Justin Nnaemeka Okorondu [3]

[1] *Department of Biochemistry, Federal University of Technology, Owerri Imo State Nigeria.*
[2] *Department of Microbiology, Federal University of Technology, Owerri Imo State Nigeria.*
[3] *Department of Environmental Technology, Federal University of Technology, Owerri Imo State Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2024, 29(01), 310–322

Publication history: Received on 30 August 2024; revised on 24 October 2024; accepted on 26 October 2024.

Article DOI: https://doi.org/10.30574/gscbps.2024.29.1.0388

## Abstract

The Comparative analysis of the Phytochemical screening and antimicrobial properties of *Hibiscus sabdariffa* Silver nanoparticles and the *Hibiscus Sabdariffa* extract was carried out.

Nanoparticle was synthesized using biological method and the synthesized nanoparticle was characterized for further confirmation using methods such as UV-Visible Spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Energy Dispersive Spectroscopy (EDX) and X-ray diffraction (XRD). Phytochemical Screening of the crude extract of *Hibiscus sabdariffa* and the nano extract was also carried out using Gas Chromatography - Mass Spectroscopy (GC/MS) in order to ascertain the biocomponents in the two extracts.

Antimicrobial properties of the crude and nano extracts were analyzed using Spectrophotometric assay**.** The result of the Uv-Visible of the nano extract showed maximum light absorption at a wavelength of 420 nm. The EDX revealed 79.52% Ag (silver) and 20.48% O (oxygen). The microscopic studies gave agglomerated spherical silver nanoparticle with average size of 65.9 nm. The XRD result shows that the silver nanoparticle exhibits a polycrystalline face centered cubic (FCC) structure with an average crystallite size of 65.9 nm. The GC/MS result of the crude extract showed a total of fifteen (15) bio compounds with 4-amino-1,2 naphtoquinone (33.8%) being the highest and hexadecenoic acid, methyl ester (0.36%) being the least in abundance. The GC/MS result of the nano extract showed a total of fifteen (15) organic compounds with Cis-vaccenic acid (46.3%) being the highest and Carbomethoxy vinyl methylamine and 6-octadecanoic acid (0.93%) being the least in abundance. The antimicrobial properties of the crude and nano extracts showed that nano extract exhibited improved antimicrobial potency against E.coli and Salmonella typhi compared to the crude extract.

**Keywords:** Antimicrobial properties; *Hibiscus sabdariffa*; Phytochemical Screening; Silver Nanoparticle

## 1. Introduction

The quest for novel antimicrobial agents has become a pressing concern in the face of escalating antibiotic resistance (WHO 2017). Plants have been a rich source of bioactive compounds with antimicrobial properties, and *Hibiscus Sabdariffa* commonly known as roselle or red sorrel, is no exception (Kumar et al, 2017). This tropical plant species has been widely used in traditional medicine for its various health benefits, including antioxidant, anti-inflammatory and antimicrobial properties (Da silva et al, 2019).

* Corresponding author: Monica Mmachukwu Okorondu

Copyright © 2024Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

Recent advancements in nanotechnology have led to the development of silver nanoparticles (AgNps), which has shown enhanced antimicrobial activity compared to their bulk counterparts (Singh et al, 2018). The combination of phytochemicals from *Hibiscus Sabdariffa* with AgNps may potentially create a synergistic effect, amplifying its antimicrobial activity (Rajan et al, 2019).

Phytochemical screening of *Hibiscus sabdariffa* has revealed a diverse array of bioactive compounds, including flavonoids, phenolic acids, anthocyanins and terpenoids (Zhang et al, 2020). These compounds have been implicated in various biological activities including antimicrobial, antioxidant and anti-inflammatory effects (Sultana et al, 2017).

This study aimed to conduct a comparative analysis of the phytochemical screening and antimicrobial properties of *Hibiscus sabdariffa* silver nanoparticle and *Hibiscus sabdariffa* extract. The objectives are:

- To perform a comprehensive phytochemical screening of *Hibiscus sabdariffa* extract and *Hibiscus sabdariffa* silver nanoparticle, to identify the bioactive compounds present.
- To evaluate and compare the antimicrobial properties of *Hibiscus sabdariffa* extract and *Hibiscus sabdariffa* silver nanoparticle against various microorganisms such as *Escherichia. coli (E. coli), Salmonella. typhi (S.typhi), Staphylococcus. aureus (Staph. aureus)*.
- To investigate the potential synergistic effect of combining *Hibiscus Sabdariffa* extract with silver nanoparticles on antimicrobial activity

## 2. Material and methods

### 2.1. Extraction Materials

Flower of *Hibiscus Sabdariffa* obtained from Eke Ukwu market Owerri, Imo State, Nigeria

#### *2.1.1. Chemical /Reagents*

Absolute Ethanol, Deionized water, Silver Nitrate (Koch – Light Laboratories England), Chloroform, Diethyl Ether, Hexane, all of analytical grade from Merck Germany.

All other chemicals and reagents used were from varied sources and of analytical grade.

#### *2.1.2. Glass Wares*

Measuring Cylinder, Filteration Funnel, Cornical Flask, Beakers,

#### *2.1.3. Equipment/ Apparatus*

Digital pH meter (Labtech, India), Incubator, UV-visible spectrophotometer model D20 (Bausch and Laumb, Germany), Digital spectrophotometer model 390 (Turner®, USA), Rotary microtome, Digital Camera (Minolta, Japan), Hot air oven (Gallenpkam, England), Water bath (Grant, England), Digital weighing balance-Mettler PT 320 (Mettler-Wagen, Switzerland), Rotary shaker (Marrienfeld, Germany), Vaccum dessicators, Deep freezer (Freshpoint FDF-196),Bench centrifuge (Clay adams, USA), Automatic micro-pipettes (TECO® diagnostics, USA), No 1 Whatman Filter paper, Magnetic Stirer (Searchtech Instruments, British Standards), Muslin Cloth, Gc-Ms 2010 QP Schimazu Japan. Rotary evaporator (Buchi Rotavapour –Switzerland),

#### *2.1.4. Culture Media*

Nutrient Agar (NA), Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMBA) and MacConkey Agar used for the characterization and identification of the test organisms were prepared according to manufacturer's specification.

### 2.2. Methods

#### *2.2.1. Preparation of Plant Extract*

Flower of Hibiscus sabdariffa obtained from Eke Ukwu market Owerri, Imo State, Nigeria were picked and removed of debris, dried at room temperature. The dried leaves were ground to fine powder using a mill (BL-335Kenwood) and stored in airtight container. 400g of the powdered flower were soaked in 2.0L, 80% ethanol. The whole set up was left to stand for 4 days with occasional agitation. They were filtered through a qualitative filter paper (no 1: Whatman,

England). The crude leaf solution was rotor evaporated at 490C (Buchi Rotav2pour, Japan) and the extract was obtained and stored in airtight container, kept in a refrigerator for further analysis.

*2.2.2. Preparation of Nano Extract*

The method of Jayachandran et al, (2021) was used with slight modifications.

50 gram of the powdered Hibiscus sabdariffa flower were soaked in 250ml deionized water and heated in a water bath at 60 0 C for one hour. It was allowed to cool, filtered with a muslin cloth and re-filtered gradually with filter paper. 1M Silver Nitrate is added to the filtrate and heated again while stirring with a magnetic stirrer for 30 minutes. Solution is cooled and centrifuged at 1000 rpm for 15 minutes. The sediment was washed twice with deionized water and evaporated to dryness. Sample is stored for further analysis.

*2.2.3. Gc-Ms Analysis*

Gc-Ms analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) and the conditions are as follows: Temperature programming from 80 – 2000C held at 800C for I minute, rate 50C/min and at 2000C for 20 minutes. FID Temperature of 300 0C, injection temperature of 250 0C, carrier gas is Nitrogen at a flow rate of 1 cm3/min, split ratio 1: 75. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5 kv and sampling rate of 0.2 seconds. The Mass Spectrum was also equipped with a computer fed Mass Spectra data bank, HERMCE Z 233 M-Z centrifuge Germany was used.

To identify the unknown phytochemical components, present in the samples, their individual mass spectral peak value was compared with the database of National Institute of Science and Technology 2014. Then identification was done by comparing the unknown peak value and chromatogram from GC-MS against the known chromatogram peak value from the NIST Library database. Subsequently, the details about their molecular formula, molecular weight, retention time and percentage content were also obtained.

*2.2.4. Characterization of Nano extract*

Characterization of the nano extract was carried out using these five methods: Uv-Visible Absorbance Spectroscopy, Energy Dispersive Spectroscopy (EDX), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD).

Uv-Visible Absorbance Spectroscopy

Uv- Vis spectroscopy was performed by a GenesysUv-vis spectrophotometer (prove 300) with a serial number (2130315791) and a software version of ISW 1.5.1. the absorption spectrum was recorded from 200nm to 900nm. The samples were measured during different time intervals between 0 and 6 hours and after 24 hours of incubation. The mixtures were stored at room temperature in quartz cuvettes and in a day-night light condition in between measurements. Also, blanks (without AgNps) of an instrument detection limit( IDL) and an Limit of blank ( LB) medium mixed in the ratio of 1:1 with the solvent specific for the stabilization type of the AgNps were measured. However, a visual analysis of the sample was performed in parallel.

Scanning Electron Microscopic Analysis

To determine the detailed morphological features of the synthesized AgNps, a Scanning Electron Microscopy (SEM)(JEOL.JSM-6380LV) (20kv) was used. The specimen used for the analysis was prepared by dispersing the powdered AgNps onto a SEM holder followed by gold (Au) coating aimed at enhancing the conductivity

Transmission Electron Microscopic Analysis

The transmission electron microscopy (TEM) was carried out for the confirmation of the shape of the particles and diffraction pattern. The powdered sample was loaded on carbon – coated copper grids by drop cost techniques and analyzed by HR TEM Model Teenai G2STWin (200kv). The size of the AgNps was calculated using the Scherrer equation:

$L=K\lambda/\beta\cos\theta$; L= Nano Crystal, λ (Lambda) = wavelength; β (beta) = peak; K = shape factor (which ranges from 0.62 – 2.08 taken at 0.59); θ = taken at 2θ in the pattern.

Energy Dispersive Spectroscopic Analysis

The reduced AgNps were assayed for their purity and energy content by the energy dispersive x-ray spectroscopy with model EDS (JSM – IT500, Joel, Boston, MA, USA). The sample was dried on a copper grid coated with carbon

X-Ray Diffraction Analysis

The synthesized silver nanoparticle was centrifuged at 10,000 rpm for 15 minutes and the pellets were redispersed in sterile double distilled and centrifuged at 10,000 rpm for 10 minutes. The purified pellets were dried at 500C in an oven and analysed by X-ray diffraction unit (XRD) (Pan Analytical, X-pert pro, Netherland). The X-ray diffraction (XRD) measurement of silver nanoparticles synthesized by leaf extracts was carried out using Cu-Kα radiation source in scattering range m(2θ) of 10-70 on the instrument operating at a voltage of 45 KV and a current of 40 mA. The presence, crystalline nature, phase variety and grain size of synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy. The particle size of the prepared samples was determined by using Scherrer's equation as follows:

$$D = K\lambda/\beta \cos \theta$$

Where D is average crystalline size, β is line broadening in radians (full width at half maximum of the peak in radians), λ is wavelength of X-ray while θ is braggs angle. K is constant (geometric factor = 0.94)

*2.2.5. Antimicrobial Analysis*

The antimicrobial analysis of Hibiscus Sabdariffa crude and nano extracts were carried out using two methods: well in agar method and spectrophotometric assay.

*2.2.6. Microscopic Identification of Bacteria isolates*

Pure cultures of foodborne bacteria previously isolated from ready-to-eat pork meat were further subjected to routine microbiological screening to ascertain their purity, viability and identities was done using standard procedures. The characterization and identification of the isolates was done following the procedures below:

Preparation of media and diluents

Bacteriological media such as Nutrient agar (NA), Salmonella Shigella agar (SSA), Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMBA) and MacConkey agar used for the characterization and identification of the test organisms were prepared according to manufacturer's specification (Sharma, 2000; Cheesbrough, 2000).

Characterization and identification of microbial isolates

Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manuals. The identities of the isolates were cross-matched with reference to standard manuals for the identification of bacteria (Beishir, 1987; Buchannan and Gibbon, 2000).

Microscopic characterization

Gram Staining Test

The Gram staining technique was used for the bacterial isolates as described by Cheesbrough (2000). A smear of the isolate was made on grease free glass slide with a drop of water and allowed to dry. The smear was fixed by mild heating, flooded with crystal violet and allowed to stand for 30 seconds. The crystal violet was rinsed off with water; Lugol's iodine was added and allowed to stand for 30 seconds. This was washed off with water and holding the slide on a tilt with a clothes pin slide, was flooded with ethanol for 5 seconds to wash off stain. It was counter stained with Safranin for 10 seconds and rinsed with water. The wet slide was allowed to air dry. A drop of oil immersion was added on the slide and viewed using X40 objective lens of the microscope. Fungal species were characterized and identified by wet mount method.

Spore Staining Test

The spore stain was used to confirm the presence of spores when indicated in the Gram stain. Isolates were heat fixed on a slide and flooded with 5% malachite green. It was sterilized for 3 minutes (without allowing it to boil), dried and cooled. It was then rinsed off and stained with Safranin for 30 seconds. This was rinsed, dried with filter paper and

viewed under the microscope using oil immersion tens. The positive spores showed green while the vegetative cells were stained pink.

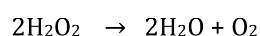
### Motility Test

This test was used to determine the motility of bacteria isolated. The test was carried out on a semi-solid agar medium in which motile bacteria swarm and gave a diffuse spreading growth. The medium was dispensed into test tubes, sterilized and allow to set in an upright position. It was then inoculated using an inoculation needle by stabbing it into the medium in the test tube. This was incubated at 37°C for 24 hours. Diffuse growth from the straight line of inoculation was recorded as positive result (Cheesbrough, 2000).

### Biochemical Characterization of Bacteria Isolates

Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to few biochemical tests described by Cheesbrough (2000) and Beishir (1987).

### Catalase Test



$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria. Catalase has one of the highest turnover numbers of all enzymes such that one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen in a second. Catalase activity can be detected by adding the substrate $H_2O_2$ to an appropriately incubated (18-24 hours) tryptic soy agar slant culture. Organisms that produces catalase enzyme, breaks down hydrogen peroxide to water and oxygen, producing bubble in the reagent drop indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to breakdown hydrogen peroxide into $O_2$ and water and are catalase negative.

### Coagulase Test

Coagulase is enzymes that clot blood plasma by a mechanism that is similar to normal clotting. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma component of blood. The only significant disease-causing bacteria of humans that produce coagulase are Staphylococcus aureus. Thus this enzyme is a good indicator of S. aureus. In the test, the sample is added to rabbit plasma and held at $37^0$C for a specified period of time. Formation of clot within four hours is indicated as positive result and indicative of a virulent Staphylococcus aureus strain. The absence of coagulation after 24 hours of incubation is a negative result indicative of a non virulent strain.

### Oxidase Test

Oxidase test is an important differential procedure that should be performed on all gram negative bacteria for their rapid identification. The test depends on the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and ∞-naphthol. This method uses N, N-dimethyl-p-phenylenediamine oxalate in which all Staphlococci specie are oxidase negative. In the presence of the enzyme cytochrome oxidase (gram negative bacteria) the N, N-dimethhyl-p-phenylenediamine oxalate and ∞-naphthol react to indophenol blue. Pseudomonas aeruginosa is an oxidase positive organism.

### Sugar Fermentation/Oxidation

This test is used to differentiate between bacteria groups that oxidize carbohydrate such as members of Enterobacteriaceae. One milliliter (1ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately under aseptic conditions transferred into duplicate tubes containing 9ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. The tubes were stab-inoculated in duplicates while two uninoculated tubes serve as control. Vaseline was used to cover one set of the duplicate tubes, one control to discourage oxidative utilization of sugar. All tubes were incubated at 37oc for 48h. After the incubation, they were observed for acid production in the culture. Yellow colouration indicates acid production in the open tubes only suggesting oxidative utilization of the sugar while acid production in the sealed tubes suggests a fermentative reaction.

Hydrogen Sulphide Production (H2S) Test

The test isolates were aseptically inoculated into a tube containing triple sugar iron agar started by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37 $^{0}$ C for 72 h and was examined daily. Black precipitation and yellow colouration was checked for. Black precipitate indicates H2S production and yellow colouration for sucrose, lactose and glucose fermentation.

Urease Test

Urease Agar slant in McCartney bottle was inoculated with the bacteria isolate at 30oC for 4 h and then overnight. A pink colour in the medium indicated a positive result.

IMViC Test

This test consists of four different test; they are Indole production, Methyl-Red test, VogesProskaeur test and Citrate utilization test. This test is specifically designed to determine the physiological properties of microorganism. They are especially useful in the differentiation of Gram-negative intestinal bacilli, particularly Escherichia coli and the Enterobacter-Klebsiella group.

Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacteria isolates were inoculated into the medium and incubated at 37°C for 48 h. At the end of incubation period, 3 drops of kovac's reagents was added and then shaken. A red colour ring at the interface of the medium denotes a positive result.

Methyl red and Voges-Proskaeur test must be considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP test, that is, MR+, VP-, or MR-, VP+.

Methyl red test was performed to demonstrate the capacity of different organisms to produce acid from the fermentation of sugar (dextrose). Methyl-red positive organisms produce a red colouration when five drops of methyl-red indicator is added into 48 h old MR-VP broth culture.

The Voges-Proskaeur test demonstrates the ability of organisms to produce acetoin from glucose metabolism. Some organisms metabolise glucose to produce pyruvic acid which is further broken down to yield Butane-diol and acetyl-methyl carbinol as an intermediate product.

Into one milliliter of the culture add one milliliter of six percent alcoholic solution of alpha-naphtol and one milliliter of 16% KOH and stand for 15-20 minutes. Development of red to pink colour is a positive test.

Citrate Utilization Test

This is one of the several techniques used to assist in the identification of Enterobacteria. Principle of the test is based on the ability of an organism to use citrate as its only source of carbon. The test was carried out using Simmon's citrate agar.

The slopes of the media were prepared in bijou bottles as recommended by the manufacturers. A sterile straight wire was used to the slope with a saline suspension of the test organisms before stabbing the butt. The bottles are incubated at 35 $^{0}$C for 48 h. Bright blue colours in the medium means positive test while no change in colour of medium indicates negative citrate test (Cheesbrough, 2000).

*2.2.7. Preparation of Test Organism*

Test organisms for standardization were sub-cultured on nutrient agar and nutrient broth at 37 $^{0}$C for 24 h. Organisms were also grown on a slant for preservation (Gotep et al., 2009). Test isolates were standardized by McFarland method and mineral salt medium. McFarland solution consists of Barium Chloride and Sulphuric Acid.

*2.2.8. Susceptibility Test*

Susceptibility of the test isolates to the extract was done by agar well diffusion assay. Four wells of 6.25 mm deep were made with a sterile cork borer on Mueller Hinton Agar previously seeded with the 24 h old standardized cultures. The wells were filled with different concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml) of the ethanol and

aqueous extracts separately. The plates were incubated for 24 hours at 370C. After 24 hours, zone of inhibition around the wells were measured and recorded in millimeters (mm).

*2.2.9. Minimum Inhibitory Concentration (MIC) Assay*

The Minimum Inhibitory Concentration Assay is a technique used to determine the lowest concentration of a particular antibiotic needed to kill an organism. This assay is typically performed on planktonic (free floating) bacterial cells. To evaluate MIC, the procedure was done according to Atlas et al. (1995).

Serial dilutions of the extracts (representing different concentrations of 500 mgml-1, 250 mgml-1, 1.25 mgml-1 and 62.5 mgml-1) was added to a growth medium (nutrient broth) in separate test tubes. These tubes were then inoculated with the standardized test isolates.

The tubes were incubated overnight. Broth tubes that appeared turbid are indicative of bacterial growth while tubes that remain clear indicate no growth. The MIC of the antibiotic/toxicant (plant extract) is the lowest concentration that does NOT show growth. This was confirmed using the spectrophotometer at 420 nm.

*2.2.10. Minimum Bactericidal Concentration (MBC) Assay*

Minimum Bactericidal Concentration is the least concentration of bacteria inhibited after 24 h incubation on nutrient agar. A loop full of the different concentrations (after spectrophotometric reading) were streaked on a freshly prepared surface dried nutrient agar and incubated overnight. Concentrations of growth after incubation was used to determine the MBC.

## 3. Results and discussion

### 3.1. Phytochemical Screening result of the crude and nano extract of *Hibiscus* Sabdariffa

**Table 1** Result of the Phytochemical Screening of the crude extract of *Hibiscus Sabdariffa*

| Peak | Compound Name | Mol. Weight | Mol. Formula | % Composition | Retention time |
|---|---|---|---|---|---|
| 1 | Butanedioic acid, diethyl ester | 174 | $C_8H_{14}O_4$ | 1.83 | 2.936 |
| 2 | Cyclohexanecarboxylic acid, 2-chloroethyl ester | 190 | $C_9H_{15}CLO_2$ | 13.52 | 3.136 |
| 3 | Butanedioic acid hydroxy, diethyl ester | 190 | $C_8H_{14}O_5$ | 3.49 | 3.547 |
| 4 | α-D-mannofuranoside | 298 | $C_{13}H_{24}B_2O_6$ | 10.09 | 3.713 |
| 5 | Succinic acid, ethyl hexyl ester | 230 | $C_{12}H_{22}O_4$ | 2.58 | 4.216 |
| 6 | Benzaldehyde, 2-fluoro-3,4-dihydroxy | 156 | $C_7H_5FO_3$ | 1.57 | 5.479 |
| 7 | Aceticacid, 2 acetyl-5-methyl-isoxazolidin-5-methyl ester | 201 | $C_9H_{15}NO_2$ | 8.04 | 5.834 |
| 8 | 4-amino-1,2 naphtoquinone | 173 | $C_{10}H_7NO_2$ | 33.82 | 6.239 |
| 9 | 4-Trifluoromethyl benzamite | 189 | $C_8H_6F_3NO$ | 12.88 | 6.559 |
| 10 | 2H-1-Benzopyran-2-one,7-amino-4-trifluoromethyl | 229 | $C_{10}H_6F_3NO_2$ | 0.89 | 6.822 |
| 11 | Pthalic acid, isobutyl 2-methylpent-3-yl ester | 306 | $C_{18}H_{26}O_4$ | 0.46 | 7.142 |
| 12 | Hexadecenoic acid methyl ester | 270 | $C_{17}H_{34}O_2$ | 0.36 | 7.394 |
| 13 | Palmitic acid, vinyl ester | 282 | $C_{18}H_{34}O_2$ | 4.33 | 7.731 |
| 14 | 9,12-octadecanoic acid, methyl ester | 294 | $C_{19}H_{34}O_2$ | 0.48 | 8.240 |
| 15 | 9,12-octadecanoic acid, ethyl ester | 308 | $C_{20}H_{36}O_2$ | 5.67 | 8.543 |

The Gc/Ms phytochemical result of the crude extract of *Hibiscus Sabdariffa* leaf shows a total of fifteen bio compounds which has 4-amino-1,2 naphtoquinone (33.82%) occurring highest while hexadecenoic acid methyl ester (0.36%)

occurs least with their molecular weight ranging from 173 – 308 and their percentage composition ranging from 0.36 – 33.82%.Majority of these biological compounds are quinones, fatty acid methyl esters (FAME), glycosides, benzamides and so on which are responsible for the plants antioxidant, anti-inflammatory, anticancer, antimicrobial activities and cardiovascular health benefits. This finding is in line with the findings of Kumar et al, 2019 who also observed majority of these phytochemicals in *Hibiscus Sabdariffa* crude extract.

**Table 2** Result of the Phytochemical Screening of the nano extract of *Hibiscus Sabdariffa*

| Peak | Compound Name | Mol. Weight | Mol. Formula | % Composition | Retention time |
|---|---|---|---|---|---|
| 1 | Methylene chloride | 84 | $CH_2CL_2$ | 1.00 | 3.536 |
| 2 | 1,16-cyclocorynan-17-oic, 19,20 didehydro, methyl ester | 322 | $C_{20}H_{22}N_2O_2$ | 1.98 | 5.508 |
| 3 | Allyl 2-ethyl butyrate | 156 | $C_9H_{16}O_2$ | 3.56 | 6.119 |
| 4 | N-(2-carbomethoxyvinyl) methylamine | 115 | $C_5H_9NO_2$ | 0.93 | 6.502 |
| 5 | Tetradecanoic acid | 228 | $C_{14}H_{28}O_2$ | 1.13 | 6.628 |
| 6 | Di-sec-butyl phthalate | 278 | $C_{16}H_{22}O_4$ | 2.93 | 7.131 |
| 7 | n-hexadecanoic acid | 256 | $C_{16}H_{32}O_2$ | 30.92 | 7.663 |
| 8 | Cis-Vaccenic acid | 282 | $C_{18}H_{34}O_2$ | 46.37 | 8.520 |
| 9 | 9-octadecanoic acid | 282 | $C_{18}H_{34}O_2$ | 1.21 | 8.937 |
| 10 | 6-octadecanoic acid | 282 | $C_{18}H_{34}O_2$ | 0.93 | 9.497 |
| 11 | 9-octadecanoic acid, 2,3-dihydroxypropyl ester | 356 | $C_{21}H_{40}O_4$ | 2.93 | 10.017 |
| 12 | 9-octadecanoic acid | 282 | $C_{18}H_{40}O_2$ | 2.06 | 10.909 |
| 13 | 17-pentatriacontene | 490 | $C_{35}H_{70}$ | 0.83 | 12.561 |
| 14 | 2,3-Dihydroxypropyl elaidate | 356 | $C_{21}H_{40}O_4$ | 2.05 | 14.475 |
| 15 | 9-octadecanoic acid 2,3-dihydroxypropyl ester | 356 | $C_{21}H_{40}O_4$ | 1.18 | 16.470 |

The result of the Gc/Ms of the nano extract recorded fifteen (15) organic compounds with Cis-Vaccenic acid (46.3%) occurring highest while N-2-carbomethoxyvinyl methylamine (0.92%) occurs lowest. Their molecular weight ranges from 84-356 while their composition ranges from 0.93-46.37%. These organic compounds contain polymers, lipids and other carbon-based molecules which are responsible for the nanoparticle anti-inflammatory, antioxidant, antimicrobial effects and its cardiovascular health support and nutritional value.

### 3.2. Characterization analysis result of the nano extract

The result of the Uv-Visible of the nano extract showed maximum light absorption at a wavelength of 420 nm. This indicates that the silver nanoparticle exhibits a strong surface plasmon resonance (SPR) at 420 nm. which could be as a result of the collective oscillations of electrons on the surface of the nanoparticles which are excited by the incident light. The initial roughness of the curve could be as a result of solvent or impurity effects which affected the lower wavelength region while the smoothing out of the curve at higher wavelengths suggests that the nanoparticle absorption dominates. This UV analysis result is related to the uv result obtained by R. Khwatr et al 2020 which reported that the formation of silver nanoparticle was confirmed by the appearance of a characteristic surface plasmon resonance (SPR) peak in the Uv-Vis spectrum. This UV result showed a strong surface plasmon resonance which confirmed that there was formation of nanoparticles.

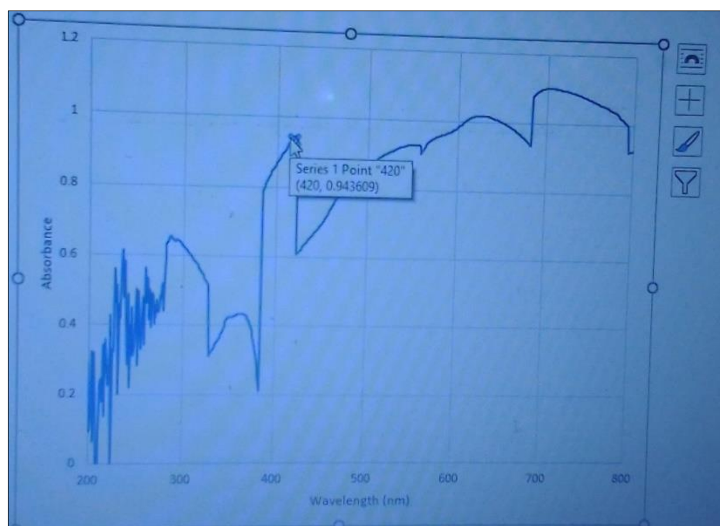



**Figure 1** Result of the characterization analysis of the nano extract of *Hibiscus sabdariffa* using UV-Visible spectrophotometer

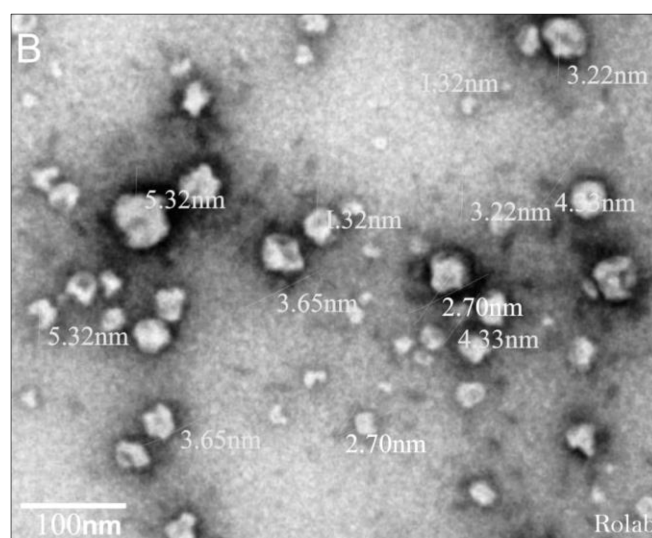



**Figure 2** 100nm resolution of TEM analysis of the nano extract of *Hibiscus sabdariffa*

The microscopic studies gave agglomerated spherical silver nanoparticle with average size of 65.9 nm. this indicates that the silver nanoparticles are in the nanoparticle range, which is consistent with the expected size range for nanoparticles. It also suggests that the nanoparticles are relatively uniform in size, which is desirable for many applications. This size of the nanoparticles can also influence their properties such as the optical properties – exhibiting strong surface plasmon resonance (SPR) absorption, which is consistent with the UV-VIS result (420 nm). it can influence catalytic activity because nanoparticle of this size can have high surface areas, making them suitable for catalytic applications. It can influence its biological interactions because nanoparticle of this size can interact with cells and biomolecules, making them suitable for biomedical applications.

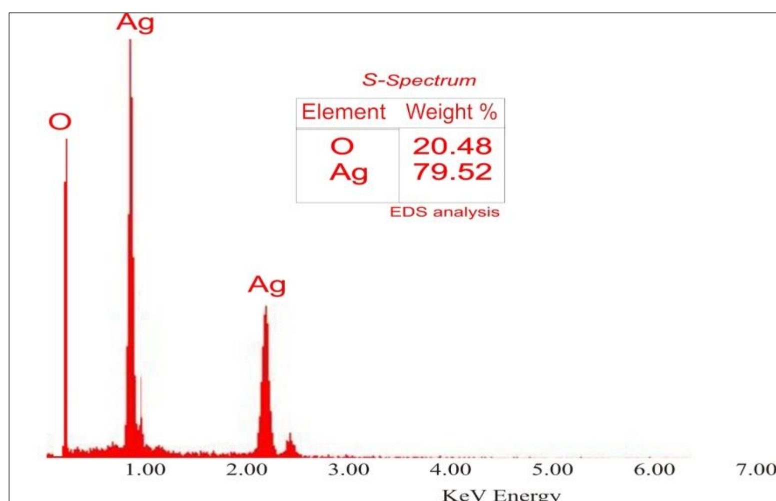



**Figure 3** Result of the Energy Dispersive Spectroscopic Analysis (EDX) of *Hibiscus sabdariffa* nano extract

The EDX revealed 79.52% Ag (silver) and 20.48% O (oxygen). This shows the presence of elemental silver. The high percentage of silver confirms the presence of silver nanoparticles. The presence of oxygen suggests that the silver nanoparticles are oxidized, possibly due to surface oxidation, silver oxide formation, residual solvent or moisture, possible silver oxide phases, surface modification or less likely contamination from environment or sample handling.

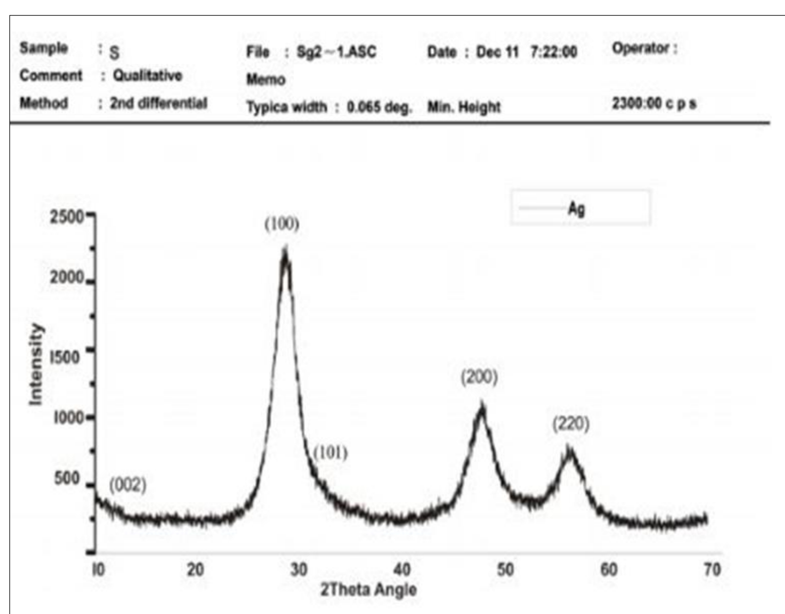



**Figure 4** Result of the X-ray Diffraction Graph of *Hibiscus sabdariffa* nano extract

The XRD result shows that the silver nanoparticle exhibits a polycrystalline face centered cubic (FCC) structure with an average crystallite size of 65.9 nm. the nanoparticles show a preferred orientation or texture as indicated by the relative peak intensities and the crystalline structure is relatively defect free with minimal lattice strain. This XRD result provided a complementary information with that of TEM analysis result.

Results of the antimicrobial analysis of the crude and nano extract of *Hibiscus Sabdariffa* using spectrophotometric assay:

**Table 1** Sensitivity of *Hibiscus Sabdariffa* Crude and Nano Extract on Test Organisms in Diameter (mm)

| Test Isolates | Sample Code | 500 mg/ml | 250 mg/ml | 125 mg/ml | 62.5 mg/ml | CIP |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | A | 17.5 | 0 | 0 | 0 | 45 |
| *Escherichia Coli* | A | 19 | 0 | 0 | 0 | 41 |
| *Salmonella Typhi* | A | 22.5 | 19 | 17.5 | 0 | 45 |
| *Staphylococcus aureus* | B | 27.5 | 32.5 | 22.5 | 25 | 42.5 |
| *Escherichia Coli* | B | 26 | 22.5 | 17.5 | 17.5 | 37.5 |
| *Salmonella Typhi* | B | 32.5 | 27.5 | 22.5 | 20 | 45 |

### 3.3. Ciprofloxacin

This table shows the antimicrobial activity of *Hibiscus Sabdariffa* crude and nano extracts against three microorganisms: *Staphylococcus aureus, Escherichia coli (E. coli*) and *Salmonella typhi.*

For the crude extract: *Staphyloccocus aureus* inhibited only at the highest concentration (500 mg/ml) with a Zone of inhibition (ZOI) of 17.5mm*. E.coli* inhibited only at the highest concentration (500 mg/ml) with a ZOI of 19mm while *Salmonella typhi* showed a dose-dependent response, with increasing ZOI at higher concentrations (22.5mm at 500 mg/ml, 19mm at 250 mg/ml and 17.5mm at 125 mg/ml).

For the nano extract: *Staphylococcus aureus* showed increased antimicrobial activity at all concentrations with a maximum ZOI of 27.5mm at 500 mg/ml*. E.coli* also showed increased antimicrobial activity at all concentrations, with a maximum ZOI of 32.5mm at 500 mg/ml. with these results, it shows that:

The nano extract exhibits higher antimicrobial activity than the crude extract indicating improved bioavailability and efficacy. This somehow corresponded with the work of Raghad, K.M et al, 2020 whose work shows that the nano extract of *Hibiscus Sabdariffa* shows increased antimicrobial activity against the crude extract.

*Salmonella typhi* is the most susceptible to both extracts, followed by *E.coli* and then *Staphylococcus aureus*.

The crude extract shows limited antimicrobial activity, only inhibiting growth at the highest concentration (500 mg/ml) for *Staphylococcus aureus* and *E.coli.*

The nano extract demonstrates a dose-dependent response, with increasing antimicrobial activity at higher concentrations.

The overall results suggest that *Hibiscus Sabdariffa* nano extract has potential antimicrobial properties, particularly against Salmonella typhi and warrants further investigation for therapeutic applications.

**Table 2** Threshold Inhibitory Concentration ($IC_{50}$) (mg/ml)

| Samples | *A* | *$R^2$* | *B* | $R^2$ |
|---|---|---|---|---|
| *E. coli* | 258.51 ± 14.60 | 0.9968 | 176.04± 15.30 | 0.9419 |
| *Staphylococcus aureus* | 207.65± 18.61 | 0.9908 | 279.31± 13.92 | 0.9912 |
| *Salmonella sp* | 309.91± 17.75 | 0.9823 | 279.34 ± 25.00 | 0.9775 |

The IC50 (half maximal inhibitory concentration) results show the concentration of *Hibiscus Sabdariffa* crude and nano extracts required to inhibit the growth of *E. coli, Staphylococcus aureus* and *Salmonella typhi* by 50%. Lower IC50 values indicate greater antimicrobial potency.

From the results on the table:

The nano extract is more potent against E. coli with a lower IC50 value.

The crude extract is more potent against *Staph. aureus* with a lower IC50 value despite the expectation that the nano should be more potent. This could be as a result of various reasons such as:

Particle size and distribution: The nano extracts particle size and distribution might not be optimal for interacting with *staph. aureus* leading to reduced potency.

Surface chemistry: The surface chemistry of the nano extract might be less favourable for interacting with *staph aureus* thus reducing its effectiveness.

Bacterial strain variability: The specific strain of s*taph. aureus* used in the test might be less susceptible to the nano extract.

Test conditions: The test conditions might not be optimal for the nano extract's activity against *staph. aureus*.

The nano extract is slightly more potent against *Salmonella typhi*, with a lower IC50 value. So in all, the nano extract exhibited improved antimicrobial potency against E. coli and Salmonella typhi compared to the crude extract

## 4. Conclusion

This study investigated the phytochemical screening and antimicrobial properties of *Hibiscus Sabdariffa* crude and nano extracts. The results revealed a diverse array of bioactive compounds in both extracts, with 4-amino-1,2 naphtoquinone and Cis-vaccenic acid being the most abundant compounds in the crude and nano extracts respectively.

Notably, the nano extract exhibited enhanced antimicrobial potency against *E.coli* and *Salmonella typhi* compared to the crude extract, suggesting that nanoscale formulation can improve the bioactivity of *Hibiscus sabdariffa*. These findings support the potential of *Hibiscus sabdariffa* nano extract as a natural antimicrobial agent for therapeutic applications.

The significant difference in the phytochemical profile and antimicrobial activity between the crude and nano extracts highlights the importance of nanotechnology in unlocking the full potential of plant based bioactive compounds.

Future studies should focus on optimizing and exploring the potential of *Hibiscus sabdariffa* nano extract in addressing antimicrobial resistance.

In all, this study contributes to the growing body of evidence on the medicinal properties of *Hibiscus sabdariffa* and underscores the potential of nanotechnology in enhancing the efficacy of natural products for healthcare applications.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The author(s) declare no conflict of interests.

## References

[1] Beishir, L.M., Finkelstein, R.A and Keith, J.M (1987). Microbiology in Practice. A self-Instruction Laboratory Course (4th ed.). Williams & Wilkins.

[2] Buchanan, R.E and Gibbon, N.E. (Eds.).(1974). Bergeys Manual of Determinative Bacteriology (8th ed.). Lippincott Williams & Wilkins.

[3] Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. (2nd ed). Cambridge University Press.

[4] Da silva, R.R., Figueiredo, E.C., De Souza, R.C., Monteiro, M.C and Menezes, E.A (2019). Hibiscus Sabdariffa: A review of its pharmacological and nutritional properties. Journal of Medicinal food 22(10): 1039-1054

[5] Jayachandran, S., Kumar, P.S., Vincent, S., Sundaramanickam, A and Senthil-Kumar, N. (2021). Green synthesis of nanoparticles using plant extracts: A review. Journal of Nanoscience and Nanotechnology, 21(10): 5319-5333.

[6] Kumar, V., Kumar, A and Pandey A. (2017). Phytochemical and pharmacological profile of Hibiscus Sabdariffa: A review. Pharmacognosy reviews, 11(22): 135-145

[7] Rajan, R., Chandran, K., Pandian, S.K., Jeyalakshmi, C and Rajesh, R (2019). Synergistic effect of phytochemicals and silver nanoparticles against microorganisms. Journal of Pharmaceutical analysis 9(3): 187-196.

[8] Singh, P., Kumar, Y., Kumar, A., Kumar, K and Kumar, M. (2018). Silver nanoparticles: Synthesis, properties and applications. Journal of nanosciences and nanotechnology, 18 (11): 7493-7517

[9] Sultana, N., Alam, M.A., Haque, M.A., Jahan, R and Rahmatullah, M (2017). Biological activities of Hibiscus Sabdariffa extracts. Journal of Pharmaceutical Sciences and Research. 9(10): 1441-1453.

[10] World health organization (2017). Global action plan on healthy lives and well-being (GLAP): Antibiotic resistance. WHO/EMP/AMI/2017.1

[11] Zhang, Y., Liu, X., Wang, Y., Chen, F and Huang, X (2020). Phytochemical analysis of Hibiscus Sabdariffa. Journal of Food Science and Technology 57(2): 541-552.

[12] Atlas, R.M., Parks, L.C and Brown, A.E (1995). Minimum Inhibitory Concentration (MIC) assay. In Handbook of Microbiological Media (pp. 721-728). CRC Press.

[13] Gotep, J., Mohan, M and Kumar, V. (2009). Growing of microorganisms on a slant agar. *Microbiology*,81(5): 635-642.