# Antioxidant and Antimicrobial Potentials of *Nicotiana glauca* Graham Leaves Extracts and Synthesized Silver Nanoparticles: A Phytochemical Approach

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## ABSTRACT

*Nicotiana glauca* is a medicinal plant that belongs to the genus *Nicotiana*, traditionally used for the treatment of many diseases. This study aims to screen the phytochemical content of leaf extracts of *Nicotiana glauca*, synthesize silver nanoparticles using the extracts, and evaluate their antioxidant and antimicrobial activities. The leaf samples were collected, air-dried, and ground into powder. The leaf powder was macerated with distilled water, methanol, n-hexane, and chloroform to extract the phytochemicals. Phytochemical analysis was performed using standard methods. Synthesis of silver nanoparticles was achieved by mixing a 3 mM silver nitrate solution with the plant extract, and the synthesized silver nanoparticles were characterized by X-ray diffraction and scanning electron microscopy. The antioxidant activities of the extracts and the synthesized silver nanoparticles were evaluated by the DPPH scavenging assay, while the in vitro antimicrobial activities were evaluated using the agar disc diffusion method against selected bacterial and fungal strains. The results of the phytochemical analysis indicated the presence of alkaloids, saponins, flavonoids, terpenoids, tannins, phenolics, steroids, and glycosides. The results of the antioxidant activity evaluation of AgNPs, methanol extract, chloroform extract, and n-hexane extract showed that they possess remarkable antioxidant activities. The antioxidant activity analysis also indicated that percentage inhibition and IC50 were dose-dependent. Synthesized silver nanoparticles showed the highest antioxidant activity with an IC50 value of 78 µg/mL, while the methanol extract gave an IC50 value of 170 µg/mL. The results of the antimicrobial activity evaluation showed that the plant extract and the synthesized silver nanoparticles exhibited antimicrobial activities. The highest zone of inhibition observed was 16.33±1.155 mm for synthesized silver nanoparticles and 15.33±1.155 mm for the plant extract. The lowest zone of inhibition observed was 9.67±0.577 mm for synthesized silver nanoparticles and 7.33±0.577 mm for the plant extract. Generally, the plant extracts and synthesized silver nanoparticles exhibited strong antioxidant and antimicrobial activities. Further studies should be conducted on the phytochemical constituents, antioxidant, and antimicrobial activities of this plant.

**Keywords:** Antimicrobial; Antioxidant; *Nicotiana glauca*; Phytochemical; Silver nanoparticle.

## 1. INTRODUCTION

Medicinal plants have been used as a source of medicine for human and animal diseases for a long time [1]. Ethiopia is one of Africa's 6th plant-rich countries, with around 60% of the plants being indigenous and most of them having medicinal properties [2]. In Ethiopia, only 10% of medicinal plant species are cultivated today, but a more significant number are left under wild stands threat [3]. Now a day medicinal plant treatments are still used for many health problems because they are safe, less toxic, economical, and a reliable key natural resource of drugs all over the world [4].

Plants contain different chemical components, such as phytochemicals, essential oils, seed oils, and others, which are important for various scientific applications and

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Received: 19/03/2024 Accepted: 18/07/2024.
DOI: https://doi.org/10.35516/jjps.v18i1.2180

pharmaceutical uses [5]. Phytochemicals are bioactive chemicals that are produced by plants. They are considered secondary metabolites because plants need them in small amounts. In addition, they are naturally synthesized in all parts of the plant body: bark, leaves, stem, root, flower, fruits, and seeds [6]. Plants are rich in many secondary metabolites that have been found to demonstrate antimicrobial, antioxidant, and anti-infectious properties [7]. Phytochemical screening of medicinal plants is important in discovering new sources of therapeutically and industrially important compounds [8].

One of the current problems in treating infectious diseases is the development of antimicrobial resistance. Microbial resistance against antibiotics is the ability of microorganisms to tolerate the effects of drugs that inhibit their growth. Microorganisms changed as a result of responding to drugs is the main reason for the resistance [10]. Searching for other ways to overcome the antimicrobial resistance of secondary metabolites is one alternative.

The Indigenous people have a long history of using plants for medicinal purposes. Despite the increasing acceptance of traditional medicine in Ethiopia, this rich indigenous knowledge is not sufficiently documented. Documentation of plants used as traditional medicines is needed so that the knowledge can be preserved, and the utilized plants can be conserved and used sustainably. In addition, the studies on the therapeutic value of plants are not that well done [9].

An antioxidant is the activity of many vitamins, minerals, and other phytochemicals. Antioxidants are used to protect the body against the damage caused by reactive oxygen species (ROS). Reactive oxygen species (ROS) can react with the body and damage many structures in the body [11]. Reactive oxygen species (ROS) cause many psychological disorders; some common examples are atherosclerosis, heart disease, aging, diabetes mellitus, immunosuppression, nervous disorders, and others. Many types of synthetic antioxidants are produced, and various food supplements containing antioxidants are there to regulate the disorder that comes from reactive oxygen species (ROS), but these synthetic antioxidant capsules and dietary supplements are found to be less effective in many cases. To fulfill the thrust of antioxidants, many more medicinal plants contain natural antioxidants that have beneficial therapeutic potential. Some studies have shown that plants produce potent phytochemicals that serve as antioxidants. The majority of the antioxidant activity is due to the presence of phytochemicals such as flavones, isoflavones, flavonoids, anthocyanin, catechins, isocatechins, phenolic compounds, and tannins [12].

Many studies have shown that various plants are rich sources of antioxidants. Antioxidants include vitamins A, C, and E, as well as phenolic compounds found in plants such as flavonoids, tannins, and lignins. The consumption of fruits and vegetables has been linked with several health benefits as a result of their medicinal properties and high nutritional value [13].

Antioxidants have the ability to control and reduce oxidative damage caused by reactive oxygen species (ROS) in foods, by inhibiting their oxidation which ultimately increasing the shelf-life and quality of these foods. Antioxidants such as Beta-carotene, ascorbic acid, and many phenolics play roles in human health such as reducing inflammation, preventing certain cancers and even delaying aging. Due to this, increasing the consumption of fruits and vegetables which are rich in antioxidants has been recommended by many researchers throughout the world [14].

Another alternative solution for the treatment of infectious diseases is the use of nanoparticles. Silver is known as one of the elements nowadays used in modern nanotechnology. The green synthesis of silver nanoparticles (AgNPs) got the attention of many researchers because of their noted properties, such as good conductivity, catalytic activity, and antimicrobial effect [15]. The excellent activities of silver nanoparticles

depend on their size, shape, and morphology. Metals and nanoparticles have shown good antimicrobial activity. but AgNPs have shown better antibacterial, antifungal, and antiviral activity than other nanoparticles [16].

*Nicotiana glauca* is one of the medicinal plants that belongs to the family Solanaceae and used in Ethiopian traditional medicine. This plant comes from South America and is distributed throughout the world. It is invasive and mainly found on the roadside. It is a flowering plant has toxic property humans and animals. *Nicotiana glauca* has the local name as "yeareb kitel" in Amharic and is widely used by traditional healers as an antibacterial, antifungal, and antiviral. It is used to treat burns and inflammatory diseases and shows good biological activities, such as allelopathic activity [17, 18]. Although it is traditionally used as medicine, its phytochemical contents and antimicrobial and antioxidant activities are not well studied. Therefore, screening for phytochemical content and testing for the antioxidant and antimicrobial activity of this plant is very important to expose its potential medicinal properties. In addition studying the potential of this plant extracts to synthesize metallic nanoparticles is imperative. The aim of this study is phytochemical analysis, and antioxidant activities and antimicrobial activities evaluation of *Nicotiana glauca* leaf extracts, and leaf extract-mediated synthesized silver nanoparticles were evaluated.

## 2. MATERIALS AND METHODS

### *Description of the Study Area*

The plant leaf samples were collected from Eteya town, Arsi zone, Oromia Regional State, Ethiopia. Eteya is the administrative center of Hetosa Wereda located about 170 km southeast from the capital, Addis Ababa. The geographical coordinates of Eteya town are $08^0$ 08ˈN, $39^0$ 14' E/ 8.133°N 39.233°E with an elevation range from 1500 to 4170 meters above sea level. The area covers 937 square km. It has 31% humidity, and its annual temperature ranges from 10-22 $^0$C.1/ 8.133°N 39.233°E The area' has bimodal rainfall occurring from March to April is a short rain season and from July to October is a long rain season [19].

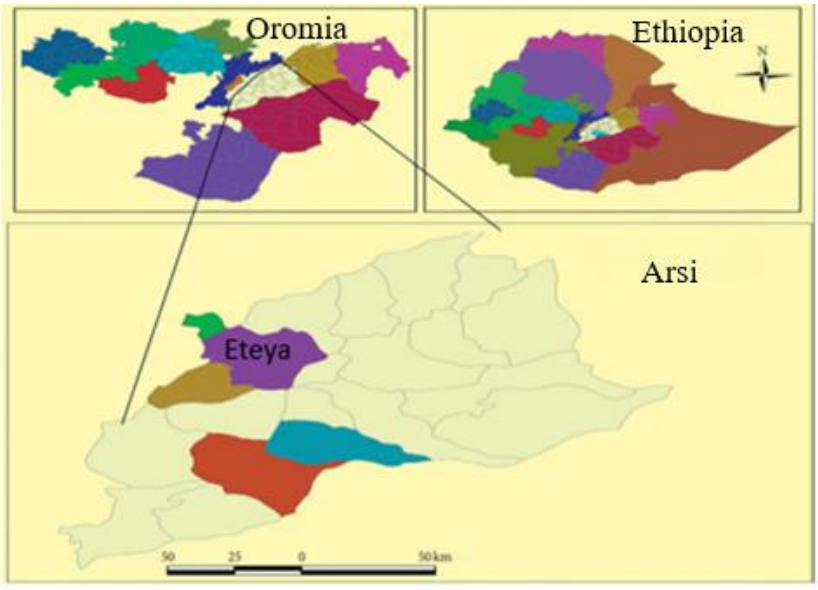



**Figure 1. Maps of the study area [20]**

***Plant materials collection and identification***

The plant materials of *Nicotiana glauca* were collected in January 2023 from fields and identified by botanist in the National Herbarium, Addis Ababa University. Voucher number (TG001) was given to the plant sample and the sample was stored in the National Herbarium, Addis Ababa University.

**Preparations of the extracts**

The collected leaves were washed with tap water to remove dirt and impurities and dried at room temperature in the shade for three weeks, The dried leaves were ground into powder using an electric grinder, and the leaf powder was weighted using an electronic balance and was kept in a flask with paper labeling. The leaf powder was weighted to 100 g using an electronic balance, and it was soaked (macerated) in 1000ml distilled water, methanol, n-hexane, and chloroform separately at room temperature for 72 hours with an electrical shaker at 120rpm. The crude extracts were filtered separately using Whatman No.1 filter paper. Then the filtrates were evaporated using a rotary evaporator, and the dried extracts were stored until used.

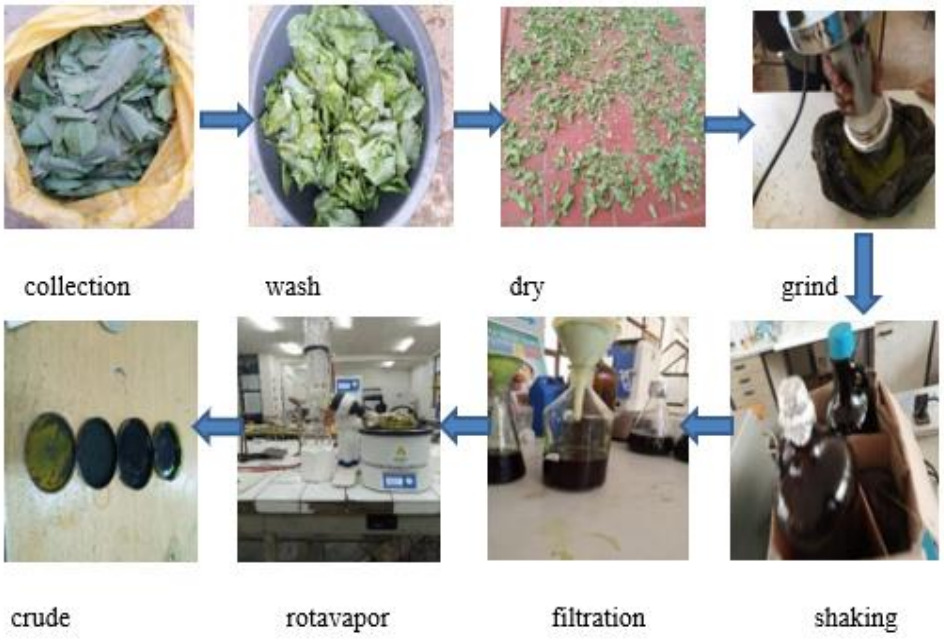



**Figure 2. The extraction processes**

**Preparation of Silver Nitrate Solution**

Silver nitrate solution was prepared by dissolving appropriate amount of silver nitrate (0.11466g) in 225 ml of double distilled water. The final concentration of the solution was made at 3mM.

***Synthesis of silver nanoparticles using leaf extracts***

The silver nitrate solution (3mM) and distilled water leaf extract were mixed in a 3:1 ratio, (225 ml of silver nitrate was mixed with 75 ml of plant extract). The mixture was heated to 60 °C for 1 hour using a magnetic stirrer. Before heating, the mixture was greenish black, any color change was recorded. The color of the mixture was changed gradually to brown which indicated the synthesis of silver nanoparticles (conversion of $Ag^{+}$ to $Ag^{0)}$. Then, the mixture was centrifuged for 10 minutes at 13000 rpm. The synthesized silver nanoparticles settled at the bottom were washed three times with double distilled water and ethanol, dried in the oven for 24 hours at 50 °C and stored

for characterization and further activity [21].

**Characterization of synthesized silver nanoparticles**

The synthesized silver nanoparticles were characterized (size and morphology were determined) by SEM (Scanning Electron Microscope) and X-RD (X-ray diffraction) (SHIMADZU Corporation Japan) 7000 X-ray diffractometer. UV


**Figure 3. Silver nanoparticle synthesizing process**

***Phytochemical screening***

The qualitative Phytochemical analysis of the extracts was done with the following standard methods to determine the presence or absence of alkaloids, saponins, flavonoids, terpenoids, tannins, phenolics, steroids, and glycosides [22-28].

**Test for alkaloids**

The presence of alkaloids in the leaf extracts was tested by Wagner's test. 2 ml of the extract was added to a test tube, and it was treated with a few drops of Wagner's test reagent. The formation of a reddish-brown precipitate or turbidity shows the presence of alkaloids.

**Test for saponins**

Foam test: 5 ml of the extract was mixed with 20 ml of distilled water, and it was shaken in a graduated cylinder for 15 minutes. The formation of about 1 cm layer of foam indicates the presence of saponins.

**Test for terpenoids**: 5 ml of each extract was added to the test tube, and 2 ml of chloroform and 3 ml of concentrated sulphuric acid were added. The color change to red brown indicated the presence of terpenoids.

**Test for flavonoids**: 2 ml of extract was treated with a few drops of sodium hydroxide solution. Then it was observed in the form of an intense yellow color.

**Test for tannins**

Lead acetate test: 2 ml of extract was added to the test tube, and a few drops of 1% lead acetate were added. The formation of a yellowish precipitate was indicated by the

presence of tannins.

**Test for phenolics**

Ferric chloride test: 1 ml of the extract was added to the test tube, and a few drops of freshly prepared ferric chloride were added. The formation of a bluish-black color implies the presence of phenolic compounds.

**Test for steroids**

Liebermann test: 1 ml of the extract was dissolved in 2 ml of chloroform into the test tube, and 2 ml of acetic anhydride was added to the test tube. Then two drops of sulphuric acid were added. Finally, the color was changed into red, then blue, and finally green.

**Test for glycosides**

Small amounts of extract dissolved in 1 ml of distilled water, then 1 ml of sodium hydroxide was added to the test tube, and a yellow color was formed. This color change indicated the presence of glycosides.

***Antioxidant activity test***

The *in vitro* antioxidant activity evaluation of the leaf extracts and synthesized silver nanoparticles was done by testing their DPPH radical-scavenging activity using spectrophotometer (UV-VIS Shimadzu) as described before [29] with slight modification. That is, 1 ml of 1mM stock solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was prepared in methanol. The test solutions were prepared in different concentrations (50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL by dissolving in dimethyl sulfoxide and put in different test tubes at an equal volume. To these solutions, 2ml of DPPH solution was added, and the mixture was allowed to react at room temperature in the dark for 30 minutes. A blank sample was prepared by adding 100 µL of dimethylsulfoxide in the DPPH solution and used as negative control. Vitamin C (ascorbic acid) was used as a standard control. The experiments were done in triplicate. After 30 minutes, the absorbance of the solutions was measured at 517 nm, and the percentage scavenging activity was calculated using the following equation:

$$\text{Percentage Scavenging} = = \frac{A0-A1}{A0} x100$$

Where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the sample extract. The IC50 values were calculated by linear regression plots. The IC50 value (concentration of sample where the absorbance of DPPH decreases 50% concerning absorbance of the control) of extracts was determined which correspond to the amount of each sample required to scavenge 50% of the DPPH free radicals.

***Evaluation of antimicrobial activities***

The antimicrobial activity of crude extracts and silver nanoparticles was evaluated on five microorganisms such as: Gram positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes), Gram negative bacteria (Pseudomonas aeruginosa, Escherichia coli)* and Fungai (*Candida albicans).* The identified test microorganisms were obtained from the Oromia regional laboratory, Adama. Ethiopia.

**Bacterial inoculum preparation**

Mueller-Hinton sterile agar plates were prepared, and the strains of bacteria: *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa,* and *Escherichia coli* were seeded on the plates. It was allowed to stay at 37°C for 24 hours. The inoculum suspension was prepared by picking several colonies of fresh Muller Hinton agar with a sterile inoculating loop and transferring the inoculum suspension into a test tube containing 5 mL of sterile Muller Hinton broth. The bacterial suspensions were adjusted to obtain turbidity visually comparable to the prepared 0.5 McFarland standards. The turbidity of the inoculum tube was adjusted to 0.5 McFarland standards ($1x10^8$cfu/mL) [30].

**Fungal inoculum preparation**

Sabouraund Dextrose (SDA) sterile agar plates were prepared, and the strain of fungus: *Candida albicans* was inoculated on the prepared plate. It was allowed to stay at 37°C for 48 hours. The inoculum suspension was prepared with a sterile inoculating loop and transferred inoculum suspended into a test tube containing 5 mL of Sabouraund Dextrose broth (SDB). The fungal suspension was

adjusted to obtain turbidity visually comparable to the prepared 0.5 McFarland standards. The turbidity of the inoculum tube was adjusted to 0.5 McFarland standards ($1x10^6$ cell/spores) [30].

**Antimicrobial test**

The standard disc diffusion method was used to evaluate the antimicrobial activities of leaf extracts and synthesized sliver nanoparticles. This method was used to test for the antimicrobial activities of the extract by measuring the zone of inhibition against the test organisms. Pure colonies of each test microorganism were diluted (0.1 ml) in sterile saline and the diluted test organisms were spread (swabbed) on Muller-Hinton agar (MHA) plates for bacteria and Sabouraund Dextrose (SDA) for the fungus by cotton swabs and allowed to dry. The extract was diluted by DMSO to make 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml concentrations. The silver nanoparticles were prepared in different concentrations: 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml. Sterilized filter paper disks (6mm in diameter) were impregnated with appropriate concentration of each plant extract and silver nanoparticles. The disks (made from Whatman No.1) were allowed to absorb the plant extracts and silver nanoparticles. The disks containing the plant extract and nanoparticles were transferred onto the surface of the seeded agar plates by using sterile forceps. The plates were kept at room temperature for 2 h for diffusion, and then it was incubated for 24 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Dimethylsulphoxide (DMSO) was used as a negative control. Ciprofloxacin and Ketoconazole were used as positive controls for bacteria and fungi, respectively. The growth was compared with the reference as well as the control [31].

**Determination of minimum inhibition concentration (MIC)**

To determine the minimum inhibitory concentration of the extracts and silver nanoparticles, the previously described method was used [32]. Plant extracts serial dilutions were prepared from 200 mg/ml of the plant extract and 10mg/ml of silver nanoparticles using DMSO. The serial dilutions prepared for extracts were 100 ml, 50 ml, 25 ml, 12.5 ml, and 6.25 ml, and the serial dilutions prepared for silver nanoparticles were 10 mg/ml, 5 mg/m 2.5 mg/ml, 1.25 mg/ml, and 0.625 mg/ml. Five milliliters of sterile Muller-Hinton Broth and Sabouraund Dextrose Broth were prepared for bacteria and fungus, respectively. The test tubes were inoculated with 10µl of the adjusted inoculum bacteria and fungi suspension with their final inoculum sizes of $5\times10^5$ CFU/ml and $2.5\times10^4$ CFU/ml respectively. The test tubes were incubated at 37°C ± 1°C for 24 hr and observed for any visible microbial growth. The minimum concentration of the extract with no visible growth was considered as MIC value.

**Determination of MBC and MFC**

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined after the determination of the MIC of bacterial and fungi. This was done by sub-culturing a sample from a test tube, yielding a negative microbial growth on Muller-Hinton Broth and Sabouraund Dextrose Broth for bacteria and fungi respectively. Then the broths were incubated according to growth requirement of each organism, to examine the presence or absence of microbial growth (turbidity). Finally, the lowest concentration of the extracts and silver nanoparticles that showed no turbidity (growth of microorganism) after incubation was considered as the MBC and MFC [32].

***Data analysis***

Antimicrobial activities were analyzed using SPSS software (SPSS 22 version). Descriptive statistics were used to summarize quantitative data. Comparisons between multiple numeric data were performed using one-way ANOVA and it was presented in mean±SD using tables. P values less than 0.05 were considered statistically significant. Microsoft Excel 2010 was used to analyze the antioxidant activity, and the results were presented by using tables and graphs.

## 3. RESULTS

***The yield of leaf extracts of Nicotiana glauca***

The extraction was done with four different solvents, such as methanol, distilled water, chloroform, and n-hexane. The results are shown in Table 2 below. 100g of plant powder was used in all solvents and the yield obtained from different solvent extractions were different (Table 1). The highest yield was obtained from Methanol extract, and the lowest yield was from n-hexane solvent.

**Table 1. The percentage yield of extracts in gram**

| Solvent | The initial mass of the plant | Yield of extract | Percentage (%) yield |
|---|---|---|---|
| Methanol | 100g | 12.8g | 12.8% |
| Distilled water | 100g | 7.3g | 7.3% |
| Chloroform | 100g | 6.5g | 6.5% |
| n-hexane | 100g | 3.4g | 3.4% |

***Synthesis and Characterization of silver nanoparticles***

The synthesis of silver nanoparticles using plant leaf extracts were confirmed by color change of the mixture. Before heating, the mixture was black then it was changed to dark brown color. This color change indicated the formation of silver nanoparticles due to the conversion of $Ag^{+}$ to $Ag^{0}$.

The XRD analysis was carried out to determine the crystalline nature of the particle and SEM was used to identify the shape, morphology, and size of green synthesized silver nanoparticles. The XRD pattern 00-004-0783 corresponds to the crystalline structure of silver (Ag). The pattern shows a series of peaks at specific angles (2θ) and intensities, which can be used to identify the crystal structure and orientation of the material. The first peak in the pattern appears at a 2θ angle of approximately 38.2°, which is the (111) reflection of the face-centered cubic (FCC) crystal structure of silver. The second peak appears at around 44.3°, which corresponds to the (200) reflection of the same crystal structure. The third peak appears at around 64.4°, which is the (220) reflection of the FCC structure. The other peaks in the pattern can also be indexed to the FCC structure of silver, including the (311) peak, it can be concluded that the sample is a polycrystalline silver material with an FCC crystal structure (Figure 4).

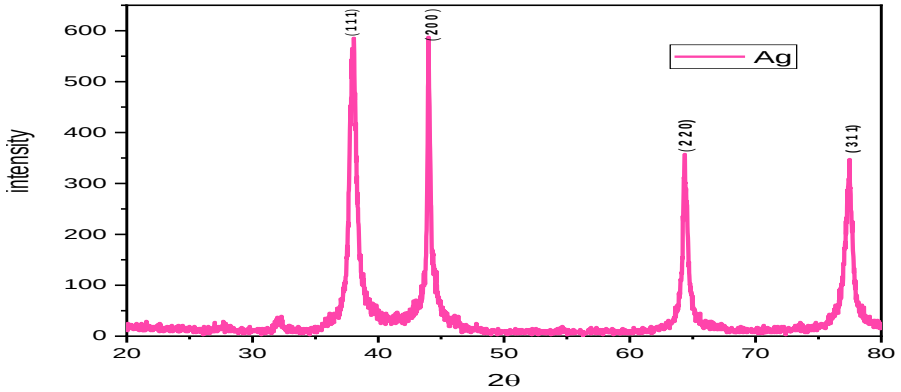



**Figure 4. X-ray diffraction (XRD) analysis of synthesized silver nanoparticles**

The morphology and shapes of synthesized silver nanoparticles were analyzed by scanning electron microscopy (SEM). The result is shown in Figure 5 below. The SEM analysis showed the agglomerated image of particles. This image is a nanomaterial, which means that the particles are very small, typically less than 100 nanometers in size. The image has a spherical shape. The fact that the image of nanoparticles is agglomerated, and aggregation appeared may be due to the secondary metabolites or salt concentration. There may be some sort of bonding or interaction between them, which could affect their properties. The surface of nanoparticles is dominated by small porous particles. Porous particles have small holes on their surface that can make them more reactive or better at adsorbing certain molecules.

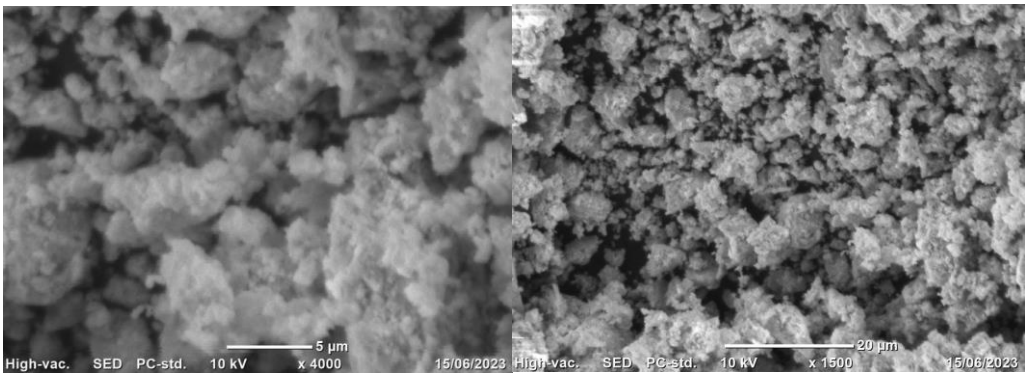



**Figure 5. SEM results of synthesized silver nanoparticles**

***Phytochemical screening of plant extract***

The phytochemical screening of *Nicotiana glauca* leaves extracts obtained by four different solvents such as methanol, distilled water, chloroform, and n-hexane was done on eight different secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, steroids, terpenoids, tannins, and glycosides. The result of the test showed the presence of all tested phytochemicals. But only alkaloids were present in the methanol extract and n-hexane extract contained only flavonoids from the tested phytochemicals. Saponins, tannins and terpenoids are found in all extracts except N-hexan. Tannins and phenolic are found only in methanol and water extracts. The results are shown in Table 2 below.

**Table 2. Phytochemical analysis of leaf extracts of *Nicotiana glauca***

| S.no | Phytochemicals compound | Test | Extracts | | | |
|---|---|---|---|---|---|---|
| | | | Methanol | D/water | n-hexane | Chloroform |
| 1 | Alkaloids | Wagner test | ++ | + | + | ++ |
| 2 | Flavonoids | Alkaline reagent Test | - | - | + | - |
| 3 | phenolics | Ferric chlorides test | + | + | _ | _ |
| 4 | steroids | Liebermann test | - | - | ++ | ++ |
| 5 | Saponins | Foam test | ++ | ++ | - | ++ |
| 6 | Tannins | Lead acetate | + | + | - | - |
| 7 | Terpenoids | Salkowski's test | ++ | + | - | ++ |
| 8 | Glycosides | Glycoside test | + | + | + | - |

The symbols ++, + and – refer to appreciable amounts, moderate to trace amount and absent amounts, respectively.

***Evaluation of Antioxidant activities***

The antioxidant activity of methanol, chloroform, n-hexane leave extracts, and synthesized silver nanoparticles was done using a DPPH scavenging assay. The results of % inhibition of ascorbic acid, crude extracts, and synthesized silver nanoparticles presented in Table 5 below. The results showed that green synthesized silver nanoparticles have the highest antioxidant activity. Next to the silver nanoparticles, methanol extract has good antioxidant activity and N-hexane extract has the lowest antioxidant activity than others (Figure 6). Generally, the antioxidant activity of the extracts and silver nanoparticles increases with increasing concentration.

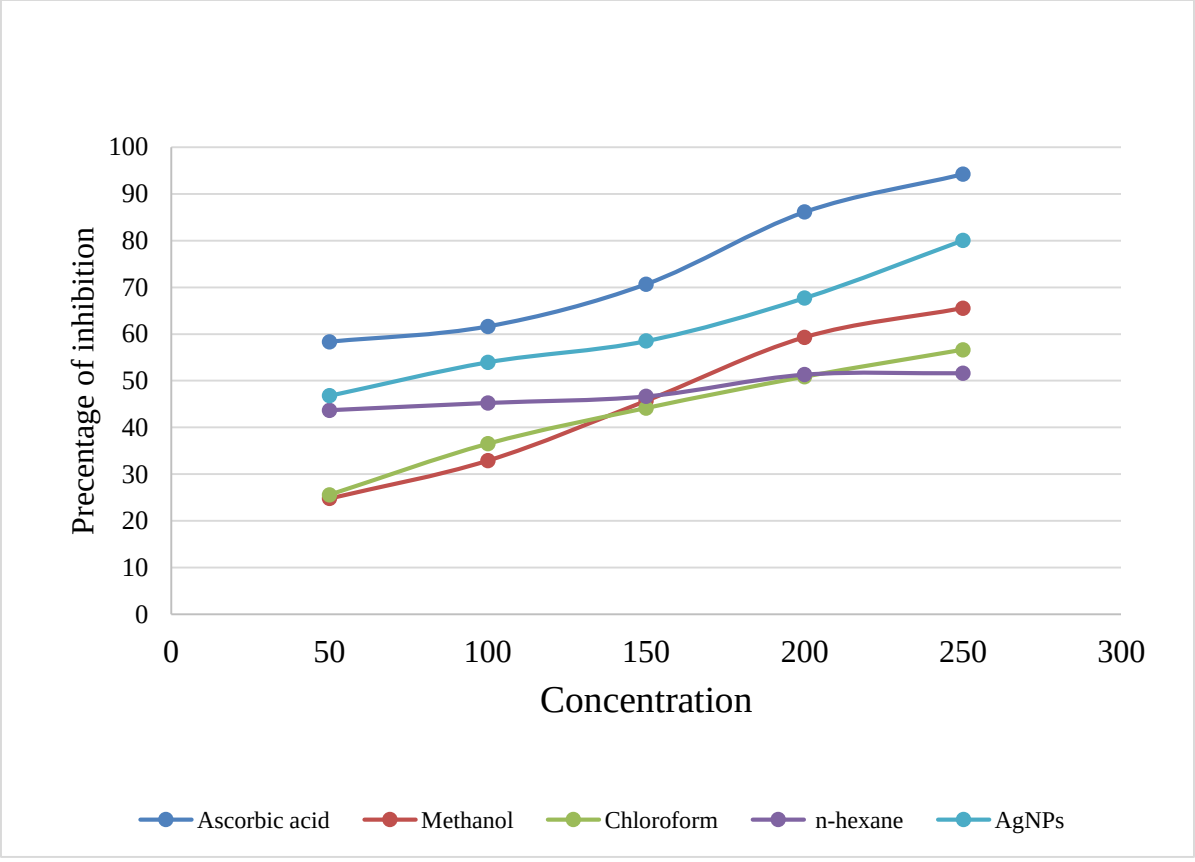



**Figure 6. The percent inhibition of ascorbic acid, plant extracts, and synthesized silver nanoparticles**

The $IC_{50}$ value of the present study implied that the ascorbic acid showed a 24 µg/mL $IC_{50}$ value. The green synthesized silver nanoparticle 78 µg/mL showed the highest activity. Methanol has an $IC_{50}$ value of 170 µg/mL, chloroform showed 197 µg/mL, and n-hexane showed a 202 µg/mL, $IC_{50}$value (Table 4).

**Table 4. The percent scavenging activity of plant extract and green synthesis silver nanoparticles**

| Concentration µg/ml | Ascorbic acid | | Methanol | | Chloroform | | n-hexane | | Silver nanoparticles | |
|---|---|---|---|---|---|---|---|---|---|---|
| | %Inhibition | $IC_{50}$ µg/mL | %Inhibition | $IC_{50}$ µg/mL | %Inhibition | $IC_{50}$ µg/mL | %Inhibition | $IC_{50}$ µg/mL | %Inhibition | $IC_{50}$ µg/mL |
| 50 | 58.34 | | 24.80 | | 25.58 | | 43.68 | | 46.80 | |
| 100 | 61.62 | | 32.91 | | 36.50 | | 45.24 | | 53.97 | |
| 150 | 70.67 | 24 | 45.70 | 170 | 44.14 | 197 | 46.64 | 202 | 58.50 | 78 |
| 200 | 86.11 | | 59.28 | | 50.85 | | 51.32 | | 67.70 | |
| 250 | 94.22 | | 65.52 | | 56.63 | | 51.63 | | 80.03 | |

***Antimicrobial activity evaluation***

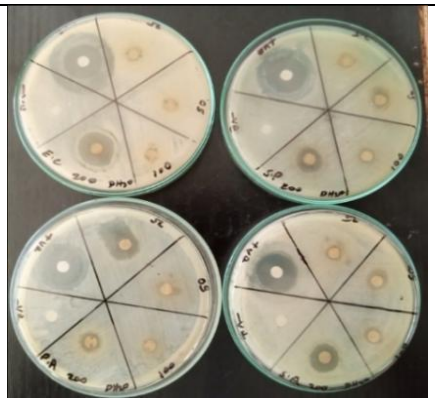
The results of antimicrobial test of the plant extracts and silver nanoparticles are given in Table 5 and Figure 8. The antibacterial activity evaluation result showed that, from the tested extracts, Methanol extract exhibited the highest antibacterial activity against *Escherichia coli* at 200mg/ml concentration followed by distilled water, Chloroform and n-hexane at concentration of 200mg/ml against *Escherichia coli, Streptococcus pyogenes* and *Staphylococcus aureus* respectively. The highest antifungal activity was exhibited by n-hexane extract against *Candida albicans.* In all the tested extracts, as concentration increases, the antibacterial activity and anti-fungal activity increases (Table 5 and Figure 8).

The synthesized silver nanoparticles also showed antibacterial and anti-fungal activity. The highest antibacterial activity and antifungal activity of the silver nanoparticles was observed on *Escherichia coli* and *Candida albicans* at 10 mg/ml respectively. Similar to the plant extracts, as concentration of the silver nanoparticles increases, the antimicrobial activity increases. When compared to the extracts, the silver nanoparticles exhibited better antimicrobial activities (Table 5 and Figure 7).

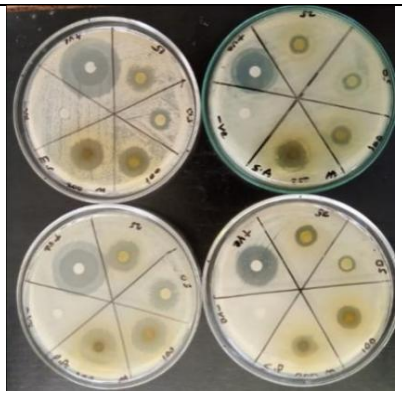
**Table 5. Zone of inhibition (mean±SD) of leaf extracts and green synthesized silver nanoparticles**

| Extracts | Concentration mg/ml | Zones of inhibition (mean±SD) in mm | | | | |
|---|---|---|---|---|---|---|
| | | *Escherichia coli* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Streptococcus pyogenes* | *Candida albicans* |
| Methanol | 200 | 15.33±1.155 | 14.00±1.000 | 14.33±0.577 | 14.00±2.000 | 12.00±1.000 |
| | 100 | 12.67±1.155 | 13.00±1.000 | 10.67±1.155 | 12.33±1.528 | 10.00±1.000 |
| | 50 | 10.00±1.000 | 8.67±1.155 | 8.33±1.528 | 10.00±1.000 | 9.00±1.000 |
| | 25 | 10.67±0.577 | 9.33±1.528 | 8.33±1.528 | 9.67±0.577 | 7.33±0.577 |
| Distilled water | 200 | 15.00±1.732 | 14.00±1.000 | 13.67±2.082 | 13.33±0.577 | 11.67±2.082 |
| | 100 | 10.67±2.082 | 11.33±1.528 | 11.33±1.528 | 10.67±1.155 | 10.33±1.528 |
| | 50 | 7.00±1.000 | 11.00±1.000 | 10.00±1.000 | 9.33±0.577 | 8.67±0.577 |
| | 25 | 7.67±1.155 | 8.33±0.577 | 8.33±0.577 | 8.00±1.000 | 7.67±0.577 |
| Chloroform | 200 | 12.67±1.155 | 13.67±0.577 | 14.00±1.000 | 15.00±1.000 | 12.33±1.528 |
| | 100 | 14.33±1.155 | 12.00±1.000 | 11.67±2.082 | 13.00±1.000 | 12.67±0.577 |
| | 50 | 12.33±1.528 | 11.000±1.000 | 9.00±1.000 | 10.00±2.000 | 12.00±1.000 |
| | 25 | 8.33±2.082 | 9.33±0.577 | 7.67±1.155 | 8.33±0.577 | 9.00±1.000 |
| n-hexane | 200 | 14.67±1.155 | 13.33±1.155 | 15.00±1.000 | 14.33±1.528 | 13.00±1.000 |
| | 100 | 10.67±1.528 | 14.33±1.528 | 11.00±1.000 | 8.67±0.577 | 10.33±0.577 |
| | 50 | 8.67±0.577 | 9.33±1.528 | 9.67±2.082 | 8.00±1.000 | 9.33±0.577 |
| | 25 | 8.00±1.000 | 8.67±1.528 | 7.67±1.155 | 7.33±0.577 | 8.67±0.577 |
| Silver nanoparticles | 10 | 16.33±1.155 | 15.67±1.155 | 15.33±1.155 | 15.00±1.000 | 15.00±1.000 |
| | 5 | 15.00±1.000 | 12.00±1.000 | 14.00±1.000 | 12.00±1.000 | 13.00±1.000 |

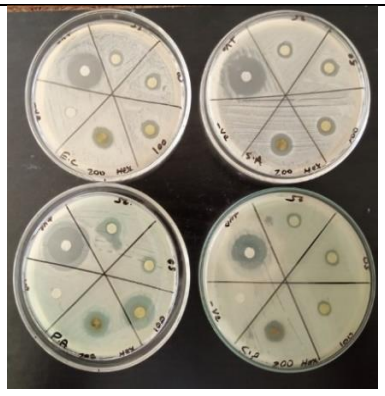
| Extracts | Concentration mg/ml | Zones of inhibition (mean±SD) in mm | | | | |
|---|---|---|---|---|---|---|
| | | ***Escherichia coli*** | ***Pseudomonas aeruginosa*** | ***Staphylococcus aureus*** | ***Streptococcus pyogenes*** | ***Candida albicans*** |
| | 2.5 | 12.00±1.000 | 10.00±1.000 | 12.67±0.577 | 10.67±1.155 | 11.67±0.577 |
| | 1.25 | 10.33±1.528 | 9.67±0.577 | 10.67±0.577 | 10.00±1.000 | 9.67±0.577 |
| Ciprofloxacin | 30 | 19.33±0.577 | 21.00±1.000 | 23.67±1.155 | 19.67±0.577 | - |
| Ketoconazole | 30 | - | - | - | - | 20.00±1.000 |
| DMSO | 1ml | 0 | 0 | 0 | 0 | 0 |



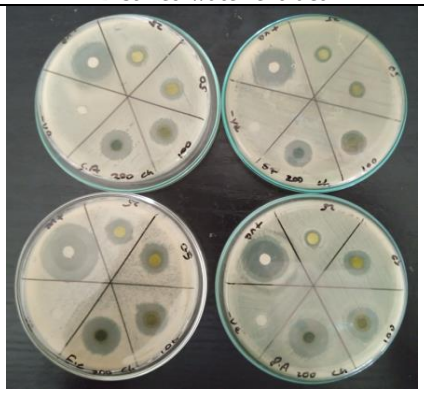
**Distilled water extract**



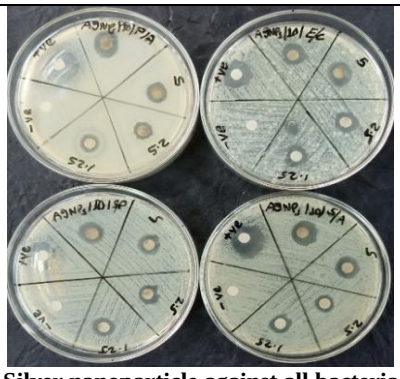
**Methanol extract**



**n-hexane extract**



**Chloroform extract**



**Silver nanoparticle against all bacteria strains**

**Antifungal activity on all extracts**

**AgNPs against *Candida albicans***

**Figure 7. Antimicrobial activities of plant extracts and green silver nanoparticles**

**Determination of Minimum Inhibitory Concentration**

The results of minimum inhibitory concentration determined using different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml) of plant extracts and (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.625 mg/ml) of synthesized silver nanoparticles against four bacteria strains and one fungus strain are presented in Table 6 below. The MIC of methanol and chloroform crude extract ranged from 12.5mg/ml to 50mg/ml for both bacterial and fungal species. The MIC value of Methanol and n-hexane ranged from 25 to 50mg/ml. Additionally, the MIC value of silver nanoparticles was 0.625mg/ml for *C. albican*.

**Table 6. The minimum inhibitory concentration (MIC) of leaf extracts of *Nicotiana glauca***

| Extracts | Minimum inhibitory concentration (in mg/ml) | | | | |
|---|---|---|---|---|---|
| | ***Escherichia coli*** | ***Pseudomonas aeruginosa*** | ***Staphylococcus aureus*** | ***Streptococcus pyogenes*** | ***Candida albicans*** |
| Methanol | 25 | 25 | 25 | 50 | 12.5 |
| Distilled water | 50 | 50 | 25 | 25 | 25 |
| Chloroform | 25 | 25 | 25 | 25 | 12.5 |
| N-hexane | 25 | 50 | 50 | 50 | 50 |
| Silver nanoparticles | 1.25 | 1.25 | 2.5 | 2.5 | 0.625 |

**Determination of Minimum Bactericidal Concentration and minimum fungicidal concentration**

The MBC and MFC were determined after the determination of the MIC of bacteria and fungi, respectively. The results of MIC showed a negative microbial growth on the surface of MHA and SDA agar plates for bacteria and fungus to examine the presence or absence of microbial growth. The lowest concentration completely inhibits the bacteria and fungus considered MBC and MFC. The results of the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) are presented in Tables 7.

**Table 7. The MBC and MFC of tested bacteria and fungi**

| Extracts | MBC and MFC (in mg/ml) | | | | |
|---|---|---|---|---|---|
| | ***Escherichia coli*** | ***Pseudomonas aeruginosa*** | ***Staphylococcus aureus*** | ***Streptococcus pyogenes*** | ***Candida albicans*** |
| Methanol | 100 | 50 | 100 | 100 | 50 |
| Distilled water | 100 | 100 | 50 | 100 | 100 |
| Chloroform | 100 | 100 | 50 | 50 | 50 |
| N-hexane | 100 | 100 | 100 | 100 | 100 |
| Silver nanoparticles | 10 | 5 | 5 | 5 | 5 |

Methanol, distilled water and chloroform crude extracts showed MBC against *S. aureus* and S. pyogenes, *E. coli, P. aeruginosa* at 50mg/ml concentration. Chloroform Showed MBC against *Streptococcus pyogenes at* 50mg/ml. The minimum bactericidal concentration against *Escherichia coli* was at concentration of 100mg/ml in all extracts. Whereas n-hexane extract inhibited all bacterial cells at 100mg/ml.

The minimum fungicidal concentration obtained by methanol and chloroform extracts were 50mg/ml. For all other extracts the MFC recorded was 100mg/ml. Moreover, Green synthesized AgNps, displayed bactericidal and fungicidal activity at low concentrations when compared with crude extracts. The synthesized silver nanoparticles inhibited killed all test bacteria and fungi at concentration of 5mg/ml except *E. coli*.

## 4. DISCUSSIONS

In this study, phytochemicals were extracted from the leaf of the *Nicotiana glauca* plant using different solvents. From the used solvents Methanol extract gave the best yield when compared to distilled water, chloroform, and n-hexane solvents. The chloroform showed better results when compared to the n-hexane. The yield obtained decreases as the polarity of the solvent decreases. This indicated that polar solvent gave excellent results when compared to the non-polar solvent. Other studies reported before also indicated methanol extracts were given the highest yield when compared to other solvents [33]. This might be due to the potential of methanol as a good solvent for extracting both polar and non-polar substance [34, 35].

The current study also indicated the presence of tested phytochemicals including alkaloids, terpenoids, steroids, flavonoids, tannins, saponins, phenols and glycosides in the crude extract of *Nicotiana glauca*. The phytochemical test done by [36], indicates that the water extract of *Nicotiana glauca* contains flavonoids and alkaloids but tannins, saponins, phenols, and glycosides were absent. In chloroform extract saponins are absent and glycosides are present. In n-hexane extract flavonoids were absent. The present findings disagree with some of the above results, in the distilled water extract alkaloids, tannins, phenols, glycosides, and saponins were presented but flavonoids were absent. In the chloroform extract, saponins were present, while glycosides were absent. In the n-hexane extract, flavonoids were present. The differences may arise from environmental factors such as mineral deficiencies, drought, cold, and disease [37]. The research findings reported by [38] indicated that the leaf extracts of *Nicotiana glauca* contain polyphenols, flavonoids, tannins, coumarins, terpenes, steroids, alkaloids, and quinones, which are consistent with the results of this study.

Phytochemicals that are present in the medicinal plant have a capacity for antimicrobial activity, anti-inflammatory activity, anticancer activity, antiallergic activity, antiviral activity, antimalarial activity, and so on [38]. In the present study, antioxidant activity was done by using the DPPH scavenging assay in different concentrations. In 250 µg/ml concentration: ascorbic acid, silver nanoparticles, methanol, chloroform, and n-hexane showed (94.22, 86.11, 70.67, 61.62, and 58.34) % scavenging activities, respectively. This showed that the plant extracts and the synthesized silver nanoparticles have antioxidant activities. The lowest $IC_{50}$ value showed the highest antioxidant activity. When we compare the samples $IC_{50}$ with ascorbic acid (24), silver nanoparticles have a 78 $IC_{50}$ value and have the highest antioxidant activity. The present study agrees with the study done before which reported that methanol extract has shown good antioxidant activity when compared to other extracts [39]. The antioxidant properties observed may be due to the presence of phenols, terpenoids, glycosides and flavonoids in the leaf of this plant. According to studies done before, phenolic and flavonoids have a high potential to scavenge free radicals [40]. This might be due to the arrangement of their properties such as type of functional groups, their redox properties and number of hydroxyl groups which enhanced the antioxidant properties [41, 42]. The antioxidant activities exhibited by the leaf extract of Nicotiana glauca align with the previous findings which confirmed free radical scavenger activities in various efficacies across multiple plants [43 and 44].

The antimicrobial activity evaluation result indicated that all the extracts and the synthesized silver nanoparticles have antibacterial and antifungal activities. The polar

solvent extracts showed excellent antibacterial activity against gram-negative bacteria. According to this study, the leaf extracts and silver nanoparticles significantly exhibited higher antimicrobial activity by showing inhibition zone that ranged from 7.33±0.577mm to 16.33±1.155mm. Methanol extract has shown 15.33±1.155mm and distilled water 15.00±1.732mm against *Escherichia coli*. While the chloroform extract showed significant activity against *Streptococcus pyogenes* 15.00±1.00mm and the n-hexane extract showed better activity against *Staphylococcus aureus* 15.00±1.00mm. The antimicrobial activity potential of the extracts may be due to their phytochemical's contents. Previous studies confirmed that plant extracts having phytochemicals such as alkaloids, flavonoids. Phenols, saponins, steroids, tannins and terpenoids have antibacterial activities. These phytochemicals inhibit the growth of microorganisms by mechanisms such as altering the permeability of cell membrane, inactivating enzyme activity, inducing cell membrane disturbance/ anxiety, and cause bacterial cell lysis [45].

Generally, the silver nanoparticles exhibited better antimicrobial activity than the extracts which agrees with previous report [46]. The study done before also showed silver nanoparticles have the highest activity in gram-positive bacteria than gram-negative bacteria, [47], which disagrees with the result of this study. The n-hexane extracts 13.00±1.000 and silver nanoparticles 15.00±1.000 showed an inhibition zone against *Candida albicans*. Both n-hexane extract and silver nanoparticles showed better activity against *Candida albicans* than others. The present studies agree with the research done before [48], the polar solvent extract such as methanol and distilled water has excellent activity than nonpolar solvents. The potential of the antibacterial activity of silver nanoparticle is due to their potential to generate reactive oxygen species; their potential to denature proteins by bonding with sulfhydryl groups by $Ag^+$ ions released from AgNPs and their potential to attach on bacteria and damage them [49-50].

The present study indicated that the MIC value of methanol extract was 25 mg/ml against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus aureus* and 50 mg/ml against *Staphylococcus pyogenes*. The methanol extract of *Nicotiana glauca* showed a MIC of 12.5 mg/ml against *Candida albicans*. In distilled water extract, the MIC showed 50 mg/ml against gram-negative bacteria, 25 mg/ml against gram-positive bacteria, and 25 mg/ml against *Candida albicans*. The chloroform extract showed MIC of 25 mg/ml in all bacterial strains and 12.5 mg/ml in *Candida albicans*. The n-hexane extract showed a MIC of 25 mg/ml against *Escherichia coli* and 50 mg/ml against *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Candida albicans*. In silver nanoparticles, MIC showed 1.25 mg/ml in *Escherichia coli* and *Pseudomonas aeruginosa*, and 2.5 mg/ml in *Streptococcus aureus* and *Staphylococcus pyogenes*. The MIC was shown at 0.625 mg/ml in *Candida albicans*. The result of the study done before [51] showed that 6 mg/ml of plant extract of *Nicotiana glauca* has the potential to stop the growth of *Staphylococcus aureus* and *Escherichia coli*, this study disagrees with the present study.

The methanol extract showed MBC at the highest concentration of 100 mg/ml against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, respectively. In *Pseudomonas aeruginosa* and *Candida albicans*, the MBC and MFC showed 50 mg/ml concentration respectively. In distilled water extract, the MBC was shown at 50 mg/ml against *Staphylococcus aureus* and 100 mg/ml against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*. The MFC value was 100 mg/ml against *Candida albicans*. In n-hexane extract, the MBC showed the highest concentration of 100 mg/ml against all bacterial strains. And the MFC also showed at 100 mg/ml. The chloroform concentration showed the MBC at 100 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa* and 50 mg/ml against *Streptococcus pyogenes* and *Staphylococcus aureus.* The MFC value of n-hexane was

50 mg/ml. The silver nanoparticle showed MBC at 5 mg/ml in *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* and 10 mg/ml in *Escherichia coli.* The MFC was shown at 5 mg/ml.

## 5. CONCLUSIONS

The results of this study confirmed that the leaf extracts of the plant *Nicotiana glauca* contain alkaloids, saponins, flavonoids, steroids, terpenoids, phenolics, tannins, and glycosides. From the solvents used to extract phytochemicals, the polar solvents contain more phytochemical compounds than nonpolar solvents. The leaf extracts of this plant and silver nanoparticles synthesized using distilled water extract of *Nicotiana glauca* the highest capacity of DPPH scavenging activities. The study also concluded that the phytochemicals present in *Nicotiana glauca* had the potential of antimicrobial activity against pathogenic bacteria and fungi. The antibacterial activities of these plants were more potent in gram-negative bacteria than gram-positive bacteria. The green synthesis silver nanoparticle showed excellent antimicrobial activity than plant extracts. Generally, the plant *Nicotiana glauca* has a strong potential for antioxidant and antimicrobial activity.

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# الإمكانيات المضادة للأكسدة والمضادة للميكروبات لاستخلاص أوراق نبات نيكوتيانا غلاوكامستخلصات أوراق غراهام وجزيئات الفضة النانوية المصنعة: نهج فيتوكيميائي

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## ملخص

نبات نيكوتيانا غلاوكا هو نبات طبي ينتمي إلى جنس نيكوتيانا ويستخدم تقليديًا لعلاج العديد من الأمراض .تهدف هذه الدراسة إلى فحص محتوى المواد الكيميائية النباتية في مستخلصات أوراق نبات Nicotiana glauca، وتخليق جزيئات الفضة النانوية باستخدام المستخلصات، وتقييم أنشطتها المضادة للأكسدة والمضادة للميكروبات .تم جمع عينات الأوراق، وتجفيفها في الهواء، وطحنها إلى مسحوق. تم نقع مسحوق الأوراق في الماء المقطر، الميثانول، الن-هكسان، والكلوروفورم لاستخراج المواد الكيميائية النباتية .تم إجراء التحليل الكيميائي النباتي باستخدام الطرق القياسية .تمت عملية تخليق جزيئات الفضة النانوية عن طريق خلط محلول نترات الفضة بتركيز 3مللي مولار مع مستخلص النبات، وتم توصيف جزيئات الفضة النانوية الناتجة باستخدام حيود الأشعة السينية والتصوير المجهري الإلكتروني الماسح .تم تقييم الأنشطة المضادة للأكسدة للمستخلصات والجسيمات النانوية الفضية التي تم تصنيعها بواسطة اختبار DPPHللكشف عن الجذور الحرة، وتم تقييم الأنشطة المضادة للميكروبات في المختبر باستخدام طريقة انتشار الأقراص في الأجار ضد سلالات بكتيرية وفطرية مختارة .نتيجة التحليل الكيميائي النباتي أشارت إلى وجود القلويدات، والصابونينات، والفلافونويدات، والتربينويدات، والتانينات، والفينولات، والستيرويدات، والجليكوسيدات .أظهرت نتائج تقييم نشاط مضادات الأكسدة لجزيئات الفضة النانوية، ومستخلص الميثانول، ومستخلص الكلوروفورم، ومستخلص الن-هكسان أن لديها أنشطة مضادة للأكسدة جيدة بشكل ملحوظ .أظهر تحليل نشاط مضادات الأكسدة أيضًا أن نسبة التثبيط و IC50كانتا معتمدتين على الجرعة. أظهرت الجسيمات النانوية الفضية المصنعة أعلى نشاط مضاد للأكسدة بقيمة IC50تبلغ 78ميكروغرام/مل، وأعطى مستخلص الميثانول قيمة IC50 تبلغ170 ميكروغرام/مل .أظهرت نتائج تقييم الأنشطة المضادة للميكروبات أن مستخلص النبات والجسيمات النانوية الفضية المُصنَّعة لهما أنشطة مضادة للميكروبات .أعلى منطقة تثبيط تم ملاحظتها كانت 16.33±1.155مم لجزيئات الفضة النانوية المصنعة و 15.33±1.155مم لاستخراج النبات .أدنى منطقة تثبيط تم ملاحظتها كانت 9.67±0.577مم لجزيئات الفضة النانوية المصنعة و 7.33±0.577مم لاستخراج النبات .بشكل عام، تمتلك مستخلصات النباتات والجسيمات النانوية الفضية المُصنَّعة أنشطة قوية مضادة للأكسدة ومضادة للميكروبات .يجب إجراء مزيد من الدراسات على المكونات الكيميائية النباتية، والأنشطة المضادة للأكسدة، والأنشطة المضادة للميكروبات لهذه النبتة.

**الكلمات الدالة:** مضاد ميكروبي، مضاد أكسدة، نيكوتيانا غلاوكا، مركب نباتي، جزيئات الفضة النانوية.

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تاريخ استلام البحث 2024/07/18 وتاريخ قبوله للنشر 2024/03/19.