Phytochemical Analysis, Antioxidant, and Antimicrobial Activities of *Nicotiana glauca graham* Leaves Extracts and Leaf Extract Mediated Synthesized Silver Nanoparticles

Tsion Guta

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

June, 2023

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Declaration

I declare that this thesis entitled "**Phytochemical Analysis, Antioxidant, and Antimicrobial Activities of *Nicotiana glauca graham* Leaves Extracts and Leaf Extract Mediated Synthesized Silver Nanoparticles**" is my own work and has not been submitted to any university for a similar purpose.

The references used in this thesis are duly recognized by proper citations.

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Name of student Signature Date

Advisor Recommendation

I, the advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled "**Phytochemical Analysis, Antioxidant and Antimicrobial Activities of *Nicotiana glauca graham* Leaves Extracts and Leaf Extract Mediated Synthesized Silver Nanoparticles**", prepared under my guidance by Tsion Guta and submitted in partial fulfillment of the Requirements for the master's degree in science in Biotechnology.

Therefore, I recommend the submission of the thesis for review and evaluation.

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Advisor/supervisor Signature Date

Advisors Approval Sheet

This is to certify that the thesis entitled "**Phytochemical Analysis, Antioxidant and Antimicrobial Activities of *Nicotiana glauca graham* Leaves Extracts and Leaf Extract Mediated Synthesized Silver Nanoparticles**" is submitted in partial fulfillment of a degree master's in biotechnology, the graduate program of applied Biology and developed by Tsion Guta. Hereby the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of this thesis.

Major advisor	Signature	Date
_____	_____	_____

Approval of the Board of Review

We, the undersigned, members of the board of examiners of the thesis by Tsion Guta have read and evaluated, Thesis entitled "**Phytochemical Analysis, Antioxidant and Antimicrobial Activities of *Nicotiana glauca graham* Leaves Extracts and Leaf Extract Mediated Synthesized Silver Nanoparticles**" and examined the candidate during the open defense. Therefore, this is to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Biotechnology.

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Dr. Ketema Tafess Tulu		13-07-2023

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ABBREVIATIONS AND ACRONYMS

AgNPs	Silver nanoparticles
ANOVA	Analysis of variance
DMSO	Dimethylsulphoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DNA	Deoxyribonucleic acid
FCC	Face centered cubic
IC_{50}	Half maximal inhibitory concentration
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MHA	Muller-Hinton agar
MHB	Muller-Hinton broth
MIC	Minimum inhibition concentration
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SDA	Sabouraund dextrose agar
SDB	Sabouraund dextrose broth
SD	Standard deviation
SEM	Scanning electron microscopy
SPSS	Statistical Package for the Social Sciences
TB	Tuberculosis
XRD	X-ray diffraction
WHO	World Health Organization

ABSTRACTS

The Nicotiana glauca belongs to the genus Nicotiana and it is traditionally used for the treatment of many diseases. This study aims to screen the phytochemicals present in the leaf extracts, to evaluate the antioxidant and antimicrobial activities of leaf extracts and leaf extract-mediated synthesized silver nanoparticles. The leaves of Nicotiana glauca were collected from Eteya town and the plant material was identified at National Herbarium, Addis Ababa University. The powder of the leaves was macerated with distilled water, methanol, n-hexane, and chloroform. Silver nanoparticle synthesis was prepared by mixing using 3mM silver nitrate solution and leaf water extract and it was characterized by X-ray diffraction and scanning electron microscopy. The phytochemical analysis was done using standard methods. The antioxidant activities were evaluated by 2,2-diphenyl-1-picrylhydrazyl scavenging assay and the in vitro antimicrobial activities evaluation were done by using the agar disk diffusion method against selected bacterial and fungal strain. The result of phytochemical analysis implied the presence of alkaloids, saponins, flavonoids, terpenoids, tannins, Phenolics, Steroids, and glycosides. The antioxidant activity of silver nanoparticles methanol, chloroform, and n-hexane was (94.22%, 86.11%, 70.67%, 61.62%, and 58.34%). In the 2,2-diphenyl-1-picrylhydrazyl assay, green synthesis silver nanoparticles have an Ic_{50} value of 78 µg/ml, and methanol extract gave an Ic_{50} value of 170 µg/ml. The results of the antimicrobial activities evaluation showed that the leaf extract and the synthesized silver nanoparticles have antimicrobial activities. The highest zone of inhibition observed was that of 16.33±1.16mm for synthesized silver nanoparticles and 15.33±1.16mm for leaf extract. The lowest zone of inhibition observed was 9.67±0.58mm for synthesized silver nanoparticles and 7.33±0.58mm for leaf extract. In conclusion, the leaf extracts Nicotiana glauca and leaf extract-mediated synthesized silver nanoparticles have good antioxidant and antimicrobial activities. Further study should be done on the phytochemical constituents, antioxidant, and antimicrobial activities of this plant.

Keywords: antimicrobial, antioxidant, *Nicotiana glauca*, phytochemical, silver nanoparticle

1. INTRODUCTION

1.1. Background of the study

Medicinal plants have been used as a source of medicine for human and animal diseases for a long time ago (Kala *et al*., 2011). Traditional practitioners have made great contributions to popularizing the role of plant medicines in human life worldwide, and they are backdated with their history for therapeutic purposes (Maridass & De Britto, 2008). 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 (Abate, *et al*., 2022). In Ethiopia, only 10% of medicinal plant species are cultivated today, but a more significant number are left under wild stands threat (Tuasha *et al*., 2018). 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 (Aneesh *et al*., 2009). Plants contain different chemical components, such as phytochemicals, essential oils, seed oils, and others, which are important for various scientific applications and pharmaceutical uses (Abate, *et al*., 2022).

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 (Maroyi, 2013). 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 (Chinemerem *et al*., 2022). Searching for other ways to overcome the antimicrobial resistance of secondary metabolites is one alternative.

Phytochemicals are bioactive chemicals that are produced by plants. They are considered secondary metabolites because plants need them in small amounts. And also, they are naturally synthesized in all parts of the plant body: bark, leaves, stem, root, flower, fruits, and seeds (Madhu *et al*., 2016). Phytochemical screening of medicinal plants is important in discovering new sources of therapeutically and industrially important compounds (Mulata *et al*., 2015). Plants are rich in

many secondary metabolites that have been found to demonstrate antimicrobial, antioxidant, and anti-infectious properties (Tadeg *et al*., 2005). These secondary metabolites present in plants have antimicrobial and antioxidant activities.

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 *(Kumar et al*., 2020). 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 (Killedar *et al*., 2014).

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 (Valko *et al*., 2006).

Antioxidants control and reduce oxidative damage in foods by inhibiting oxidation caused by reactive oxygen species (ROS), ultimately increasing the shelf-life and quality of these foods. Beta-carotene, ascorbic acid, and many phenolics play dynamic roles in delaying aging, reducing inflammation, and preventing certain cancers. Increasing the consumption of fruits and vegetables has been recommended by many researchers throughout the world, but there is a big gap (Vivekananthan *et al*., 2004).

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 *(*Ashraf *et al*., 2016). 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 (Siddiqi *et al*., 2018).

Nicotiana glauca is a medicinal plant that belongs to the family Solanaceae. It is a flowering plant. As the study showed in 1930, *Nicotiana glauca* is toxic to humans and animals (Asma Rinez, 2012). *Nicotiana glauca* has the local name as "yeareb kitel" in Amharic. *Nicotiana glauca* is widely used by traditional healers as an antibacterial, antifungal, and antiviral (Abir *et al*, 2022). It is used to treat burns and inflammatory diseases and shows good biological activities (Hassan *et al*., 2014). Tree *Nicotiana glauca* comes from South America and is distributed throughout the world. It is invasive and mainly found on the roadside (Tabana *et al*., 2015).

1.2. Statement of the problem

Medicinal plants have been used as a source of medicine since a long time ago in Eteya Town and its area. Eteya Town contains different types of medicinal plants. Among these plants is *Nicotiana glauca*, which is widely distributed, and has several traditional uses. The leaves of *Nicotiana glauca* are a solution for different diseases. But not only the leaves part other components of plants like, stem, seed, root, etc. could give good alternatives to the limited therapeutic options that exist for the treatment of different diseases (Tabana *et al*., 2015).

Medicinal plants are major components of antibiotics and are also used in different applications in modern science. Screening for phytochemical constituents and evaluation of the antimicrobial and antioxidant activities of the leaf extract and green synthesis silver nanoparticles of *Nicotiana glauca* is very important because this plant is used widely by people traditionally. But the therapeutic potential and doses of the plant have not been scientifically evaluated. As we know green synthesis of silver nanoparticles has excellent antimicrobial activity, so the present study was to evaluate the antimicrobial potential of green synthesis silver nanoparticles of *Nicotiana glauca*.

Antioxidants are very important compounds that are used by the human body to prevent damage from reactive oxygen species (ROS). Synthetic antioxidant capsules and dietary supplements are used, but they are helping the body be less effective in many cases, and synthetic antioxidant capsules also have many side effects. Instead of these synthetic antioxidants, many medicinal plants contain natural antioxidants that have beneficial therapeutic potential so identification of the antioxidant activities of plants will solve the problem (Killedar *et al*., 2014).

Currently, there is an increase in the development of antimicrobial resistance among different pathogenic bacteria and fungus species. This development of antimicrobial resistance against the existing antimicrobial agents is becoming a huge problem in controlling infectious diseases around the world and in our country. Therefore, searching for alternative antibacterial and antifungal agents such as phytochemicals, is very important. Scientists are searching for phytochemicals that have antibacterial and antifungal activities to solve the problem of antimicrobial resistance. *Nicotiana glauca* is dispersed all over the country. And enough amount of *Nicotiana glauca* has been used for the general treatment of different diseases and to solve the problem of antimicrobial resistance (Ali Alghamdi, 2021). But there are not enough scientific bases for the therapeutic actions of traditional *Nicotiana glauca* medicines, which may serve as the source for the development of more effective drugs. So, in this study, phytochemical analysis, antioxidant activities, and antimicrobial activities of *Nicotiana glauca* leaf extracts, and leaf extract-mediated synthesized silver nanoparticles were evaluated.

Research questions

1. What are the major phytochemicals that are presented in the leaf extracts of *Nicotiana glauca*?
2. Do the leaf extracts of *Nicotiana glauca* have antioxidant activities?
3. Do the leaf extracts of *Nicotiana glauca* have antimicrobial activities?
4. Do the silver nanoparticles synthesized from leaf extracts of *Nicotiana glauca* have antioxidant and antimicrobial activities?
5. What is the minimum inhibitory concentration of the leaf extracts, and leaf extract-mediated synthesized silver nanoparticles of *Nicotiana glauca?*

6. What are the minimum bactericidal concentration and minimum fungicidal concentration of the leaf extracts, and leaf extract-mediated synthesized silver nanoparticles of *Nicotiana glauca?*

1.3. Objectives

1.3.1. General objective

- To screen the phytochemical constituents and to evaluate antioxidant and antimicrobial activities of leaves extracts of *Nicotiana glauca* and leaf extract-mediated synthesized silver nanoparticles

1.3.2. Specific objectives

- To identify the phytochemical contents from leaf extracts of *Nicotiana glauca*.
- To evaluate the antioxidant activity of leaf extracts, and leaf extract-mediated synthesized silver nanoparticles of *Nicotiana glauca.*
- To evaluate the antimicrobial activities of leaf extracts and leaf extract-mediated synthesized silver nanoparticles of *Nicotiana glauca.*
- To determine the minimum inhibitory concentrations of different solvent extracts, and leaf extract mediated synthesized silver nanoparticles of *Nicotiana glauca.*
- To determine the minimum bactericidal concentrations, and the minimum fungicidal concentration of different solvent extracts, and leaf extract-mediated synthesized silver nanoparticles of *Nicotiana glauca.*

1.4. Significance of the study

This study gives information on the phytochemical constituents, antioxidant, and antimicrobial activities of leaf extracts of *Nicotiana glauca* and leaf extract-mediated synthesized silver nanoparticles. It is important to solve the problem of antibiotic resistance, and side effects of synthetic drugs, it is cheap, and affordable so, it is helping society. This finding may be used as secondary data for the researcher to carry out further investigation on this plant.

2. LITERATURE REVIEW

2.1. Medicinal plant

A medicinal plant is a plant that is used for medicinal purposes. Medicinal plants are very necessary for their uses in treatment and for providing ecological, economic, and cultural services. Since a long time ago, the world has used medicinal plants to treat diseases and fight infections. Plants have been rich sources of effective and safe medicines (Raut *et al*., 2013). As the World Health Organization reported, nearly 3.5 billion people in developing countries, including Ethiopia, believe in the ability of plant remedies and use them regularly (Teka & Maryo, 2023).

Many plants have medicinal value and are rich sources of various pharmacologically potent compounds. The therapeutic potentials of plant drugs have been used traditionally since back historical times (Bhattacharyya *et al*., 2019). About 64% of the world's population depends on traditional medicine for their healthcare (Phondani *et al*., 2016). The medicinal properties of medicinal plants come due to the presence of various bioactive compounds present in the plants like alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids (Karthikeyan *et al*., 2009). More than 80% of Ethiopian people depend on plants for their health services. Ethiopia is a home of diversity, having diverse medicinal plants as well as ethnic groups, each having different ways of preparing and utilization of medicinal plants (Karthikeyan *et al*., 2009). The traditional healer has been ignored in the past by educated and modern societies. Recently, several ethnomedicinal and ethnobotanical studies have been carried out, knowing the benefit of traditional medicine in promoting healthcare services (Wondimu *et al*., 2007).

Medicinal plants are used for various research studies. More than thirteen thousand plants have been tested for multiple medicinal properties (Teklehaymanot & Giday, 2007). Ethiopian plants have shown very effective medicinal value for some diseases of both humans and domestic animals. Thus, medicinal plants and knowledge of their use contribute to human and livestock healthcare needs in the country (Birhanu, 2015). Traditional medicine has been belaved as the most affordable and easily accessible source of treatment in the primary health care system of resource-poor communities in Ethiopia. About 80% of the population in developing countries uses traditional medicines because they cannot afford the high cost of western pharmaceuticals and for

health care, though the drugs have adverse effects like hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin, and abnormal liver function (Hailu *et al*., 2020).

2.2. Phytochemical compounds from Medicinal plant

Medicinal plants naturally contain phytochemicals within their leaves, stems, seeds, and roots that have defense mechanisms and protect from various diseases. Phytochemicals are classified into two types of compounds: primary and secondary compounds. Chlorophyll, proteins, and common sugars are included as primary compounds, and terpenoids, alkaloids, and phenolic compounds are secondary compounds (Krishnaiah *et al*., 2007). The secondary metabolites are responsible for the therapeutic activity of the plant. Because of this, the phytochemical study of medicinal plants should be carried out. Many medicinal plants were tested for secondary metabolites for their medicinal values (Siddhan & Kumari, 2014).

2.2.1. Alkaloids

Alkaloids are the main pharmacologically active compounds produced by plants, and they are metabolic byproducts derived from amino acids. Alkaloids were extracted from the different parts of the plants using different solvents such as ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous. These phytochemical components are extracted from the leaves, roots, stem bark, and fruits of medicinal plants (Ullah *et al*., 2014).

2.2.2. Flavonoids

Flavonoids are a compound that is present in medicinal plants. It consists of a large group of polyphenol compounds with a benzoyl-γ-pyrone structure and is ubiquitously present in plants. They are synthesized by the phenylpropanoid pathway. Secondary metabolites of phenolic nature, including flavonoids are responsible for a variety of various pharmacological activities (Mahomoodally *et al*., 2005).

2.2.3. Steroids

The word steroid is derived from sterol, a natural or synthetic chemically active hormone-like element. A steroid is one of a large group of chemical substances classified by a specific carbon structure. Steroid hormone is produced in mammalian female and male gonads and adrenal glands;

however, many studies also report steroid production by plants. Steroids are produced by tobacco plants. Different types of steroids extracted have the potential for bioactivities (Popova *et al*., 2020).

2.2.4. Saponins

Saponin is one of the types of phytochemicals produced in medicinal plants. It has several pharmacological values, such as lowering serum cholesterol, permeabilizing the cell membrane, and stimulating luteinizing hormone (Francis *et al*., 2002; Thakur *et al*., 2013). Saponins have a capacity for immunomodulatory potential via cytokine interplay, cytostatic and cytotoxic effects on the malignant tumor cell, adjuvant properties for vaccines as immunostimulatory complexes, and synergistic enhancement of the toxicity of immunotoxins (Bachran *et al*., 2008).

2.2.5. Tannins

Tannin prevents plants from being edible for worms and confers resistance to microbes. They have been used as food and medicine for their effects against tumors, oxidants, or microbes. Tannins do not function entirely as primary antioxidants (Serrano *et al*., 2009).

2.2.6. Phenolics compound

Phenolic is another compound produced by medicinal plants. It has been considered a good antioxidant *in vitro* and has more potent antioxidants than Vitamins C, E, and carotenoids (Prithviraj Karak, 2019). The phenolic compounds can scavenge radical species such as ROS/RNS, suppressing ROS/RNS formation by inhibiting some enzymes and regulating or protecting antioxidants (Ghasemzadeh & Ghasemzadeh, 2011).

2.2.7. Terpenoids

Terpenoids are small molecular products synthesized by plants and are the most widespread natural products. Terpenoids show significant pharmacological activities, including cancer, and also have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antihyperglycemic, anti-inflammatory, and immunomodulatory properties. Terpenoids can be used as protective substances in storing agricultural products as they are known to have insecticidal properties (Boroushaki *et al*., 2016).

2.2.8. Glycosides

Phytochemicals are organic compounds. It has many biological activities, such as antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. Phytochemicals have a major solution for the antimicrobial resistance of bacteria (Khare *et al*., 2021). Of these organic compounds, glycosides are one. Glycosides are organic compounds that are isolated from plants or animals. It plays many important roles. Many plants store chemicals in the form of inactive glycosides that are activated by enzyme hydrolysis. Important for the sugar to break off. (Nandi *et al*., 2021).

2.3. Silver nanoparticles

In modern science, nanotechnology is one alternative way to overcome antimicrobial resistance. It's the process of synthesizing particles that are in the nano range. Nanoparticles range from 1 to 100 nm. They have a large surface area to volume ratio, due to which they possess optical properties, as they are small enough to confine their electrons and produce quantum effects by which their detection becomes easy (Rautela *et al*., 2019).

As different experiments done before showed, silver nanoparticles showed good antimicrobial activity against Vibrio cholera, Salmonella typhi, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa as compared to other metals (Li *et al*., 2010). Not only has antimicrobial activity but it also has potent anticancer activity, and antitumor activity by reducing cell proliferation, promoting intracellular reactive oxygen species, initiating DNA damage, and apoptosis among cancerous tissues (Agent, 2020).

2.4. Classification and morphological features of *Nicotiana glauca*

Nicotiana glauca is a member of the Solanaceae family and a flowering plant in the *Nicotiana* genus. It's known by the common name tree tobacco. *Nicotiana glauca* is a small tree or shrub with many branches that normally grow to over 2m, but it can reach as high as 7m. It is an evergreen perennial plant that grows in open and disturbed areas. Its leaves are thick and can be up to 20cm long. It has yellow flowers about 5cm long and 1cm wide. The plant reproduces by seed. Species of *Nicotiana glauca* grow in different parts of the world and have been used medicinally by human societies (Asma, 2012).

Table 1. Scientific classification of the plant *Nicotiana glauca* (Asma, 2012).

Kingdom	Plantae
Phylum	Angiosperms
Class	Eudicots
Order	Solanales
Family	Solanaceae
Genus	*Nicotiana*
Species	*Nicotiana glauca*

Figure 1. *Nicotiana glauca* tree (Furer *et al*., 2011).

2.5. Ethnomedicinal values of *Nicotiana glauca*

Consumption of medicinal plants has been increasing over the past time as an alternative approach to improving the quality of life and maintaining good health. Medicinal plants have also been used for centuries as remedies for human diseases. Since a long time ago, people have accepted traditional medicine as an alternative form of health care, and the development of microbial resistance to the available antibiotics has led researchers to study the antimicrobial activity of plant extracts. Plants containing flavonoids, terpenoids, steroids, phenolic compounds, and alkaloids have antimicrobial activity (Latha, 2009).

Nicotiana glauca has an arthrogrypotic congenital on piglets and found significant results (Ramadan *et al*., 2018). According to a study done before, the extracts of *Nicotiana glauca* can be served as antioxidant, antimicrobial, antifungal, cytotoxic, anti-inflammatory, larvicidal, hepatoprotective, spasmolytic, anti-proliferation, and anti-tumor activities. It was demonstrated that the non-boiled *Nicotiana glauca* aqueous extract from leaves reduced total serum bilirubin levels, while the boiled and non-boiled aqueous extracts from flowers were noneffective. The desire to turn to herbal treatments comes largely from the risks of modern medicine (Shaw *et al*., 2003; Mdee *et al*., 2009).

2.6. Antioxidant activity of *Nicotiana glauca*

DPPH scavenging assay is a method used to evaluate plant extracts' antioxidant activities. DPPH is a free radical that accepts an electron or hydrogen atom to become a stable molecule. Antioxidants interact with DPPH radicals and transfer electrons or hydrogen atoms to DPPH and then neutralize its free radical character and convert it to 1,1-diphenyl-2- picryl hydrazine. The degree of discoloration indicates the scavenging activity of the extract. The reduction of the DPPH radical was determined by the decrease in its absorbance at 517 nm that was stimulated by plant extracts (Abuzaid *et al*., 2020).

Medicinal plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Boots *et al*., 2008). Several antioxidant compounds can be found in fruits and vegetables, including phenolics, carotenoids, anthocyanins, and tocopherols. Approximately 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system in positive ways such as treating cancer and harmful diseases. Plants can produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Alothman *et al*., 2009). The presence of total phenolics and flavonoids in *Nicotiana glauca* is responsible for many biological activities, including free radical scavenging assay (Tabana *et al*., 2015).

Methanolic extract from the flowers and leaves of *Nicotiana glauca* with the largest amount of phenolic compound presented the best overall performance, i.e., the best antioxidants, and the highest anti-inflammatory activities. The results obtained from various investigations support the traditional use of plant species as antioxidants and anti-inflammatory agents. The extracts from the

leaves of *Nicotiana glauca,* which are the parts traditionally used, do have a good antioxidant and anti-inflammatory effect (Gutiérrez *et al*., 2014).

2.7. Antimicrobial activity of *Nicotiana glauca*

The study done by different researchers gives the scientific basis for the traditionally claimed use of the medicinal plant for the treatment of bacterial infections like infectious skin disorders, wound healing problems, antidiarrhea, and others. The antimicrobial activities of the plant might be linked with the presence of non-polar and intermediately polar bioactive secondary metabolites, including alkaloids, terpenoids, tannins, saponins, and flavonoids (Birhan *et al.*, 2018). Traditional medicinal plants are important sources of natural products for treating common infectious bacteria due to the emergence of multidrug-resistant infectious bacteria and the high cost of synthetic compounds. Unwanted side effects of certain drugs have led pharmaceutical companies to look for new therapeutic agents from alternative sources, including medicinal plants (Tuasha *et al*., 2018).

As studied by different researchers, the methanol and n-hexane leaf extracts of *Nicotiana glauca* showed good antimicrobial activity (Abdel Rahman *et al*., 2011). A study done previously showed that the leaf extract of *Nicotiana glauca* revealed more antibacterial activity than other parts of the plant. Also, phytochemicals were present in the leaves, flowers, and stems of *Nicotiana glauca* compared to its roots. The ethyl acetate extracts of *Nicotiana glauca* showed excellent activity against *Escherichia coli* when we compare them to the gram-positive bacteria *Staphylococcus aureus* (Ali Alghamdi, 2021). The plant *Nicotiana glauca* revealed better activities against antibiotic-resistant bacterial strains such as *S. enterica, L. innocua,* and *C. perfringens* (Abdel Rahman *et al*., 2011). The methanolic and distilled water extracts of *Nicotiana glauca* had a capacity for good antibacterial activities against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Streptococcus agalactiae*. The 200 mg/ml concentration of plant extracts showed a better inhibition zone than 100 mg/ml (Mohamed *et al*., 2022).

Fungi occur everywhere and are well adapted to use a wide range of substrates as their carbon, nitrogen, and energy sources. The growth of many fungi is challenging to control because of their capacity to metabolize many substances. Fungi cause severe diseases in plants, animals, and humans. Many studies have been done to screen medicinal plants for their antifungal activity to

cure many diseases that come from fungal infections. A different group of researchers has initiated antifungal programs for traditionally used plant classes of compounds from plant metabolites, such as terpenoids, saponins, phenolic compounds, flavonoids, coumarins, alkaloids, proteins, and peptides, that show antifungal activity against different fungal species (USLU *et al*., 2018). The study was done before *Nicotiana glauca* had the highest capacity to induce the in vitro growth of the tested fungal species (Asma, 2012).

3. MATERIALS AND METHODS

3.1. 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 from the capital, Addis Ababa. The geographical coordinates of Eteya town are $8^0$8’0” N, 39^0 14’0” E, with an elevation range from 1500 to 4170 meters above sea level. The area covers 937 square km (Asfaw *et al*., 2021). It has 31% humidity, and its annual temperature ranges from 10-22 ^{0}C. The Wind NE at 18 km/h. The area's bimodal rainfall occurring from March to April is a short rain season and from July to October is a long rain season (Yirga *et al*., 2018).

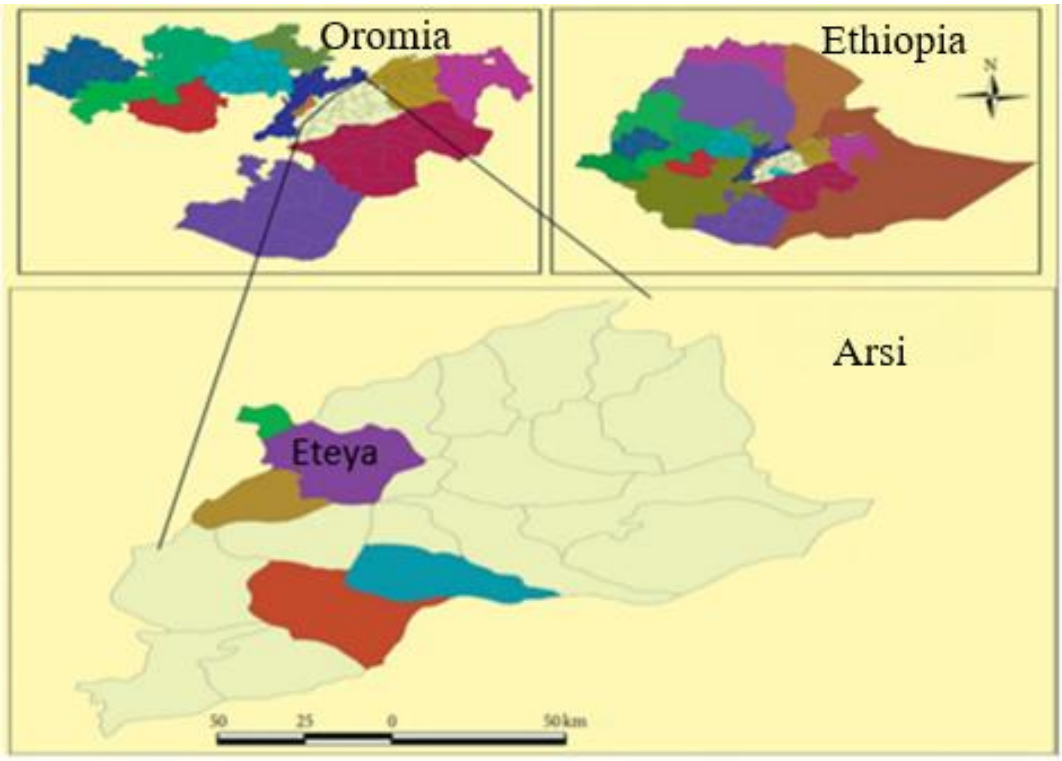


Figure 2. Maps of the study area (Asfaw *et al*., 2021)

3.2. Plant materials collection and identification

The samples of *Nicotiana glauca* were collected in January 2023 from fields around Eteya town, Hexosa wereda, Arsi zone, Oromia regional state, Ethiopia. The plant material was identified 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.

3.3. Preparations of the extracts

The 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 water, methanol, n-hexane, and chloroform separately 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 in a refrigerator until used (Birhan *et al*., 2018).

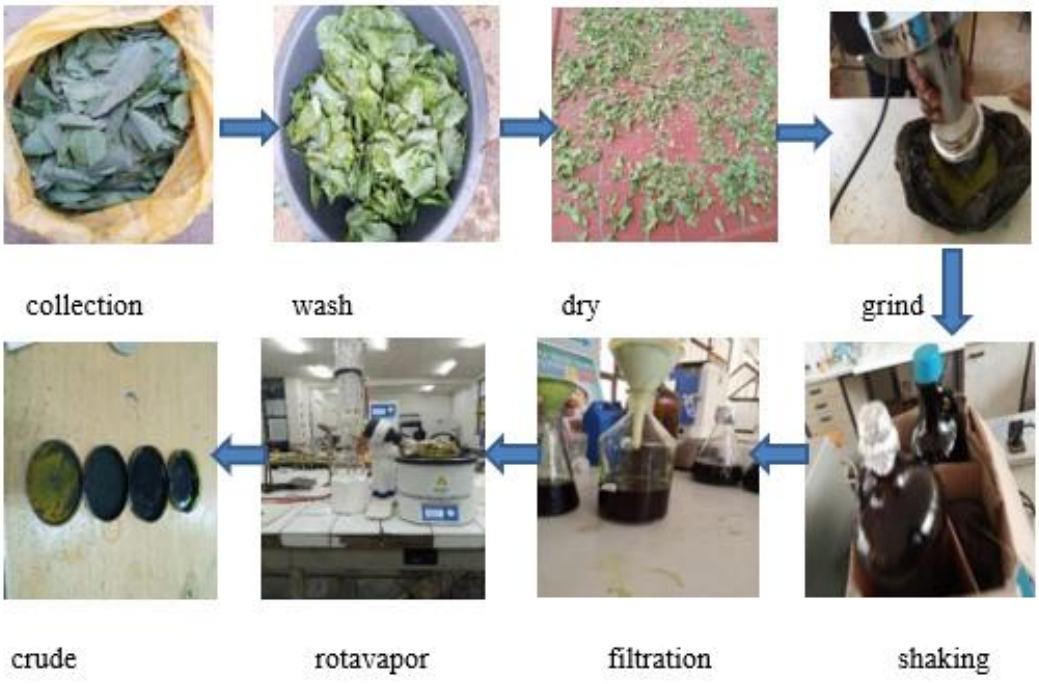


Figure 3. The extraction processes

3.4. Green synthesis of silver nanoparticles using leaf extracts

3.4.1. Preparation of plant extract

According to (Jihan *et al*, 2022) the ground leaves were weighted 30g using an electronic balance, and it was soaked in 300 ml distilled water for 72 hours in a shaking incubator at 25°C. The extract was filtered through Whatman No.1 filter paper. The filtrates were stored in the refrigerator until used.

3.4.2. Preparation of silver nitrate solution.

Silver nitrate solution was prepared by mixing 0.11466g of AgNO3 in 225 ml of double distilled water. The final concentration of the solution was made at 3mM (Aritonang *et al*., 2019).

3.4.3. Green synthesis of silver nanoparticles

The silver nitrate and 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 black, any color change was recorded. Then the mixture was centrifuged for 10 minutes at 13000 rpm. The synthesized silver nanoparticles were settled at the bottom. The synthesized silver nanoparticles were washed three times with double distilled water and ethanol. The silver nanoparticles were dried in the oven for 24 hours at 50 °C. The synthesized silver nanoparticle was stored for characterization and further activity (Christopher *et al*., 2015).

3.4.4. Characterization of silver nanoparticles

The size and morphology of synthesized AgNPs were determined by scanning electron microscope (JCM-6000plus, JEOL/EO, Japan). The biosynthesis of AgNPs was determined by using X-ray diffraction (SHIMADZU Corporation Japan) 7000 X-ray diffractometer.

Figure 4. Silver nanoparticle synthesizing process

3.5. Phytochemical screening

The qualitative Phytochemical analysis was done with the following standard methods: The methanol, aqueous, chloroform, and n-hexane extracts of the leaves of *Nicotiana glauca* were tested for the presence or absence of alkaloids, saponins, flavonoids, terpenoids, tannins, phenolics, steroids, and glycosides (Trifa *et al*., 2020).

3.5.1. Test for alkaloids

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 was produced, and this shows the presence of alkaloids (Karthikeyan & Vidya, 2019).

3.5.2. 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. A 1 cm layer of foam indicated the presence of saponins (Kumar *et al*., 2009).

3.5.3. Test for flavonoids

Alkaline Reagent Test: 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 (Trifa *et al*., 2020).

3.5.4. Test for terpenoids

Salkowski's test: 1 ml of 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 (Santhi & Sengottuvel, 2016).

3.5.5. 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 (Ukoha *et al*., 2011).

3.5.6. 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 (Karthikeyan & Vidya, 2019).

3.5.7. 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 (Auwal *et al*., 2014).

3.5.8. 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 (Longbap *et al*., 2018).

3.6. Antioxidant activity

The analysis of *in vitro* antioxidant activity was done to assess the antioxidant potential of the *Nicotiana glauca* leaf extract by using the DPPH scavenging assay. The DPPH radical-scavenging activity of the test leaf extracts was examined as previously described by (Piana *et al*., 2016). That is, 1 ml of stock solution was prepared, and different concentrations (50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL) of each plant extract, silver nanoparticle, and standard were taken in different test tubes at an equal volume. To these solutions, 2ml of a methanol solution of

DPPH (0.1 mM) was added, and the mixture was allowed to react at room temperature in the dark within 30 minutes of incubation. Vitamin C (ascorbic acid) was used as a standard control. After 30 minutes, the absorbance (A) was measured at 517 nm, and it was converted into the percentage antioxidant activity using the following equation: % scavenged.

Percentage of inhibition = (%) = $[A_0-A_1)/A_0] \times 100$, Where A_0 is the absorbance of the control reaction 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; the higher the antioxidant activity, the lower the IC_{50} value.

3.7. Evaluation of antimicrobial activities

The antimicrobial activity of crude extracts and silver nanoparticles of *Nicotiana glauca* was evaluated on the following pathogens: *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli,* and *Candida albicans*. test microorganism was taken from the Adama Public Health Research and Referral Laboratory.

3.7.1. 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 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 ($1x10^8$cfu/mL) (Adedapo *et al*., 2008).

3.7.2. 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 SDA and transferred inoculum suspended into a

test tube containing 5 mL of Sabouraund Dextrose broth (SDB) using a sterile inoculum loop. 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) (Adedapo *et al*., 2008).

3.7.3. Agar disk diffusion

The disk 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. Test organisms were diluted, and diluted inoculums (100µl) of test organisms were spread 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 nanoparticle was 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 put in the appropriate concentration for each sample. The disks (made from Whatman No.1) were allowed to absorb the samples. The disks containing the samples were transferred onto the surface of the seeded agar plates by using flamed but cooled 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 using a ruler. Dimethylsulphoxide (DMSO) was used as a negative control. 30µg/ml Ciprofloxacin and ketoconazole were used as positive controls for bacteria and fungi, respectively. The growth will be compared with the reference as well as the control (Mahomoodally *et al*., 2005).

3.7.4. Determination of minimum inhibition concentration (MIC)

The method described by (Birhan *et al*., 2018) was used to determine the minimum inhibitory concentration of the extracts. Serial dilutions were prepared from 200 mg/ml of the plant extract using 2 ml DMSO. The test tubes with 5 ml of Muller-Hinton and Sabouraund Dextrose sterile broths for bacteria and fungus, respectively, were prepared. The serial dilutions of the extract 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, and the serial dilutions for silver nanoparticles: 10 mg/ml, 5 mg/m 2.5 mg/ml, 1.25 mg/ml, and 0.625 mg/ml were added to the test tube separately. The test tubes were inoculated with further diluted 10µl of the adjusted inoculum

bacteria and fungi suspension with their final inoculum sizes of 5×10^5 CFU/ml and 2.5×10^4 CFU/ml respectively. The test tubes were incubated at 37°C ± 1°C for 24 hr. The minimum concentration of the extract with no visible growth was considered as MIC value.

3.7.5. 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 sub-culturing a sample from a test tube, yielding a negative microbial growth on the surface of MHA and SDA agar plates. Then the plates were incubated for 24hrs and 48hrs at 37°C for bacteria and fungus, respectively, to examine the presence or absence of microbial growth. Finally, the lowest concentration of the extracts that showed no bacterial and fungal cell growth after incubation was considered as the MBC and MFC (Birhan *et al*., 2018).

3.8. Data analysis

Antimicrobial activities were analyzed using SPSS software (SPSS 22 version). Descriptive statistics were used to summarize quantitative data and presented as mean±SD. Microsoft Excel 2010 was used to analyze the antioxidant activity and the results were presented by using tables and graphs.

4. RESULTS

4.1. The yield of plant extract

The extraction was done with four different organic 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, respectively. The mass of extract that was obtained was different in all four solvents. The methanol yielded 12.8g, distilled water 7.3g, chloroform 6.5g, and n-hexane 3.4g.

Table 2. 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%

4.2. Green synthesis of silver nanoparticles

The green synthesis silver nanoparticles were prepared by mixing 225 ml of silver nitrate with 75 ml of plant filtrate in a 3:1 ratio. The yield of nanoparticles was 1.529g. Before heating the mixture was black then it was changed to dark brown color. This color change indicated the formation of silver nanoparticles. 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 synthesis of 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 degrees 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 peak in the pattern can also appears at around 76.6° 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 5).

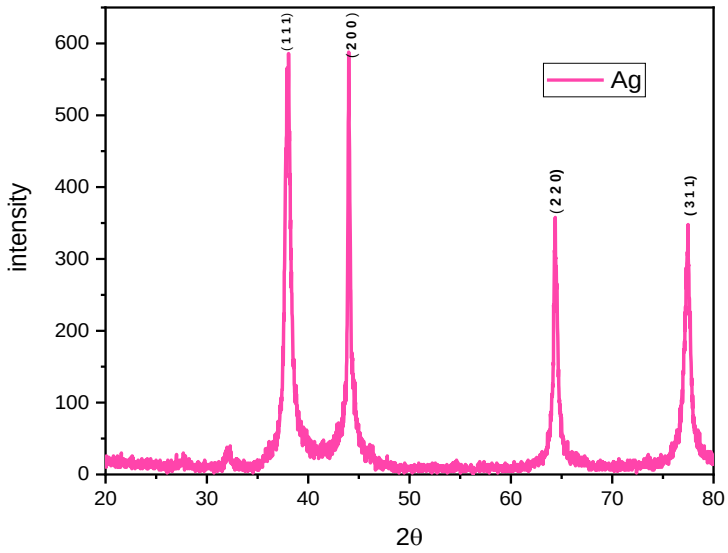


Figure 5. X-ray diffraction (XRD) analysis

The morphology, size, and shapes of synthesized silver nanoparticles were analyzed by scanning electron microscopy (SEM). The result is shown in Figure 6 below. The SEM analysis showed the agglomerated image of particles. This image is a nanomaterial, which means that the particles are very small, typically ranging from 62-84 nanometers in size. The image has a spherical shape.

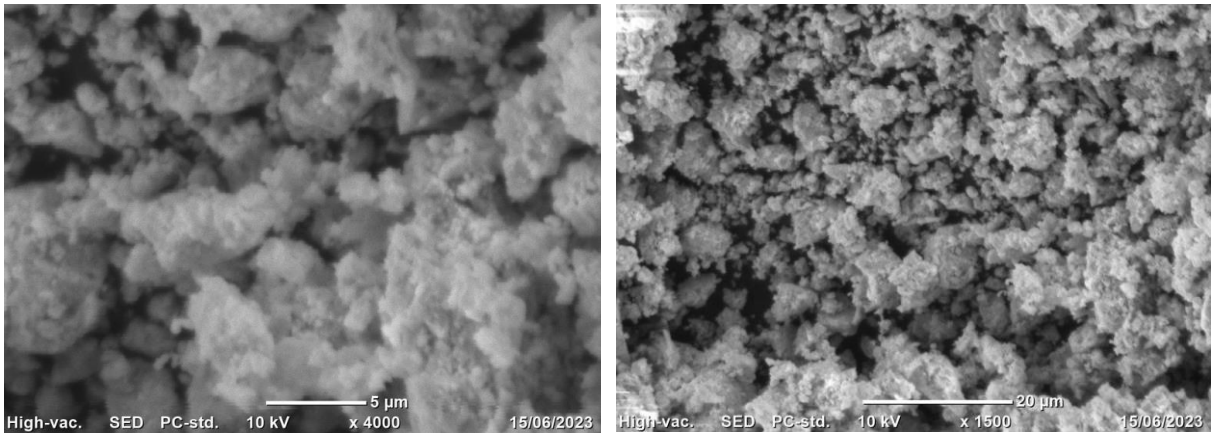


Figure 6. SEM results of silver nanoparticles

4.3. Phytochemical screening

The phytochemical screening of *Nicotiana glauca* leaves that were extracted 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 results are shown in Table 3 below.

Table 3. 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	+	+	+	_

Key ++ highly present, + present, – absent

4.4. Antioxidant activity

The antioxidant activity of methanol, chloroform, n-hexane leave extracts, and green synthesized silver nanoparticles of *Nicotiana glauca* was done using a DPPH scavenging assay. DPPH and samples were dissolved by methanol. leaf extracts, green synthesis silver nanoparticles, and the positive control ascorbic acid were prepared in different concentrations: 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml. The DPPH absorbance of 0.641 was used as a control. Ascorbic acid was used as standard. The absorbance of leaf extracts, green synthesis silver nanoparticles, and standard control were recorded at 517nm. The results were presented in Table 4 below. The

percentage scavenging activity was calculated by using the formula: % scavenged.

Percentage of inhibition= (%) = $[(A_0-A_1)/A_0] \times 100$

Table 4. The absorbance of standards, leaf extracts, and green synthesis silver nanoparticles

Concentration	Absorbance at 517nm					
	Control	Ascorbic acid	Methanol	Chloroform	n-hexane	Silver nanoparticle
50	0.641	0.267	0.482	0.477	0.361	0.341
100	0.641	0.246	0.430	0.407	0.351	0.295
150	0.641	0.188	0.348	0.358	0.342	0.266
200	0.641	0.089	0.261	0.315	0.312	0.207
250	0.641	0.037	0.221	0.278	0.310	0.128

The percentage scavenging activities of ascorbic acid, leaf extracts, and green synthesis of silver nanoparticles were calculated using (Percentage of inhibition= (%) = $[(A_0-A_1)/A_0] \times 100$). The result presented in Table 5 below showed green synthesis silver nanoparticles have the highest antioxidant activity. Next to the silver nanoparticles, methanol has good antioxidant activity. Chloroform has good activity than n-hexane. N-hexane has the lowest antioxidant activity than others (Figure 7).

Table 5. The percentage scavenging activity of leaf extracts and green synthesis silver nanoparticles

Conc	Ascorbic acid		Methanol		Chloroform		N-hexane		AgNPs	
	%I	Ic_{50}	%I	Ic_{50}	%I	Ic_{50}	%I	Ic_{50}	%I	Ic_{50}
50	58.34	24	24.80	170	25.58	197	43.68	202	46.80	78
100	61.62		32.91		36.50		45.24		53.97	

150	70.67		45.70		44.14		46.64		58.50	
200	86.11		59.28		50.85		51.32		67.70	
250	94.22		65.52		56.63		51.63		80.03	

The Ic_{50} (concentration of sample where the absorbance of DPPH decreases 50% concerning absorbance of the control) value was calculated by using the linear regression plot. The Ic_{50} value of the present study implied that the ascorbic acid showed a 24 µg/ml Ic_{50} value. The green synthesis silver nanoparticle 78 µg/ml showed the highest activity. Methanol extract has an Ic_{50} value of 170 µg/ml, chloroform extract showed 197 µg/ml, and n-hexane extract showed a 202 µg/ml, Ic_{50} value. The lowest Ic_{50} value has the highest antioxidant activity. Ascorbic acid > green synthesis silver nanoparticle > methanol extract > chloroform extract > n-hexane extract (Figure 8, Table 5).

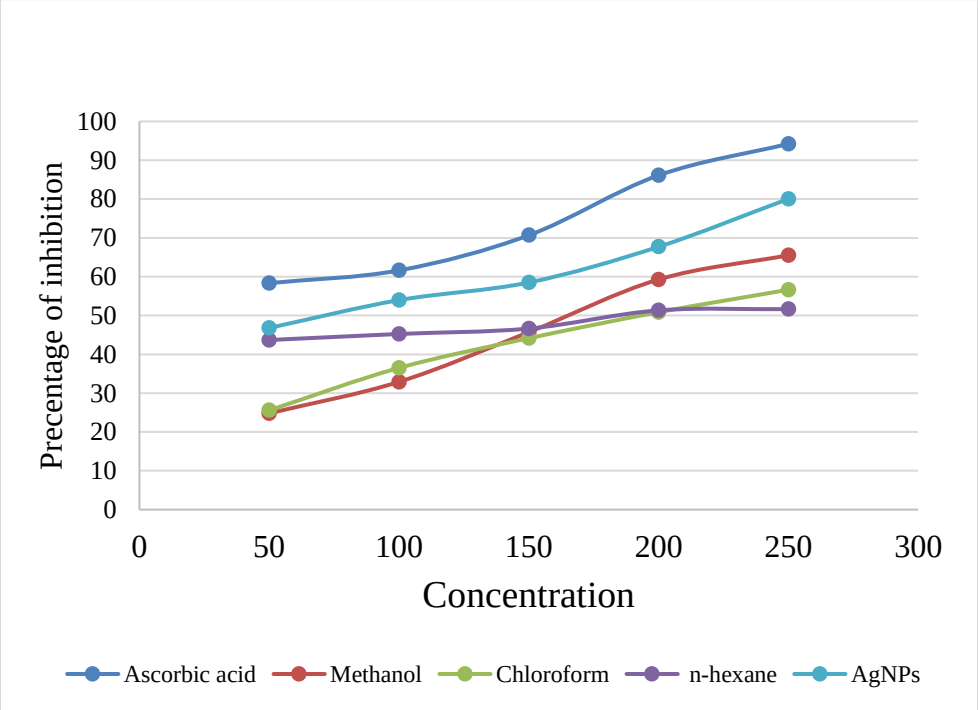


Figure 7. The percentage inhibition of ascorbic acid, leaf extracts, and green synthesis silver nanoparticles

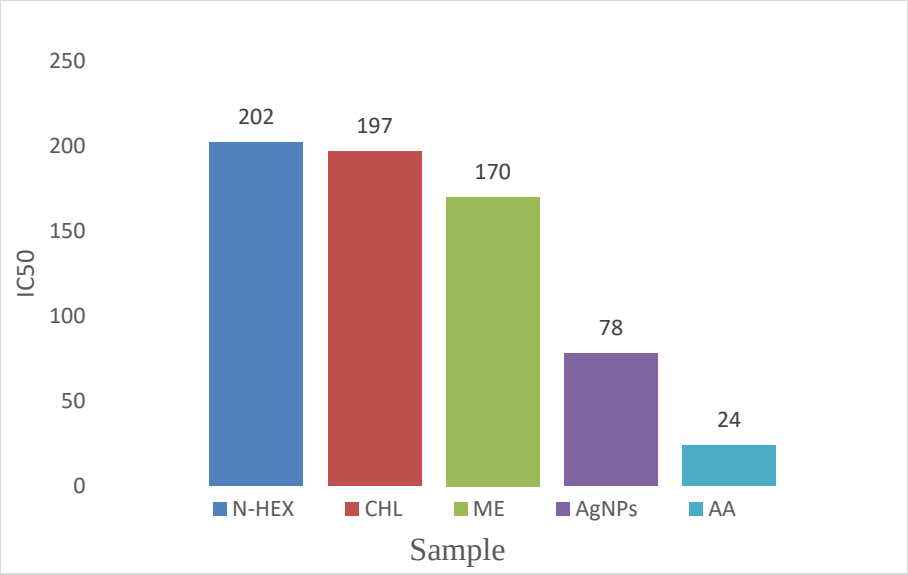


Figure 8. The Ic50 value of standard, leaf extracts, and green synthesis silver nanoparticles

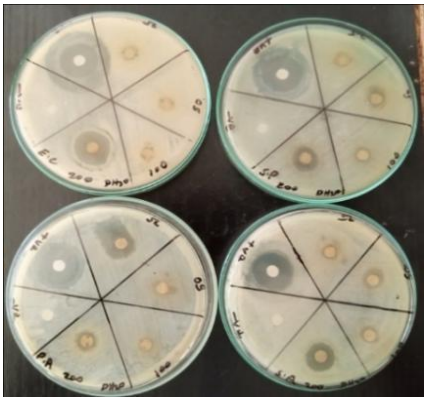
4.5. Antimicrobial activity

The antimicrobial activity of methanol, distilled water, n-hexane, chloroform extracts, and green synthesis silver nanoparticles in the leaf of *Nicotiana glauca* was tested against four pathogenic bacteria strains: *Staphylococcus aureus, Streptococcus pyogenes*, *Pseudomonas aeruginosa, and Escherichia coli*, and one fungus strain: *Candida albicans*. The extracts were prepared in different concentrations: 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml. and also green synthesis silver nanoparticles prepared in different concentrations: 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml. The 30 µg/ml ciprofloxacin and ketoconazole were used as a standard control for bacteria and fungus, respectively. The sample was dissolved using dimethylsulphoxide (DMSO). DMSO was used as a negative control. A 6mm disk was prepared from Whatman No. 1 filter paper. The plant extracts and green synthesis silver nanoparticle antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. The results were analyzed by using (SPSS 22) mean±SD. The results were shown in Table 6, Figure 9.

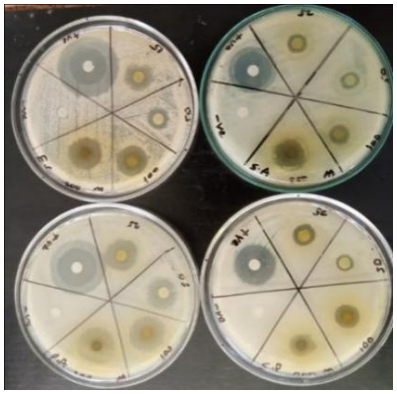
Table 6. Zone of inhibition (mean±SD) of leaf extracts of *Nicotiana glauca* and green synthesis silver nanoparticles

Extracts	Conc	Zones of inhibition (mean±SD)				
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Candida albicans*
Methanol	200	15.33±1.16	14.00±1.00	14.33±0.58	14.00±2.00	12.00±1.00
	100	12.67±1.16	13.00±1.00	10.67±1.16	12.33±1.53	10.00±1.00
	50	10.00±1.00	8.67±1.16	8.33±1.53	10.00±1.00	9.00±1.00
	25	10.67±0.58	9.33±1.53	8.33±1.53	9.67±0.58	7.33±0.58
Distilled water	200	15.00±1.73	14.00±1.00	13.67±2.08	13.33±0.58	11.67±2.08
	100	10.67±2.08	11.33±1.53	11.33±1.53	10.67±1.16	10.33±1.53
	50	7.00±1.00	11.00±1.00	10.00±1.00	9.33±0.58	8.67±0.58
	25	7.67±1.16	8.33±0.58	8.33±0.58	8.00±1.00	7.67±0.58
Chloroform	200	12.67±1.16	13.67±0.58	14.00±1.00	15.00±1.00	12.33±1.53
	100	14.33±1.16	12.00±1.00	11.67±2.08	13.00±1.00	12.67±0.58
	50	12.33±1.53	11.000±1.00	9.00±1.00	10.00±2.00	12.00±1.00
	25	8.33±2.08	9.33±0.58	7.67±1.16	8.33±0.58	9.00±1.00
n-hexane	200	14.67±1.16	13.33±1.16	15.00±1.00	14.33±1.53	13.00±1.00
	100	10.67±1.53	14.33±1.53	11.00±1.00	8.67±0.58	10.33±0.58
	50	8.67±0.58	9.33±1.53	9.67±2.08	8.00±1.00	9.33±0.58
	25	8.00±1.00	8.67±1.53	7.67±1.16	7.33±0.58	8.67±0.58
AgNPs	10	16.33±1.16	15.67±1.16	15.33±1.16	15.00±1.00	15.00±1.00
	5	15.00±1.00	12.00±1.00	14.00±1.00	12.00±1.00	13.00±1.00
	2.5	12.00±1.00	10.00±1.00	12.67±0.58	10.67±1.16	11.67±0.58
	1.25	10.33±1.53	9.67±0.58	10.67±0.58	10.00±1.00	9.67±0.58
Ciprofloxacin	30	19.33±0.58	21.00±1.00	23.67±1.16	19.67±0.58	-

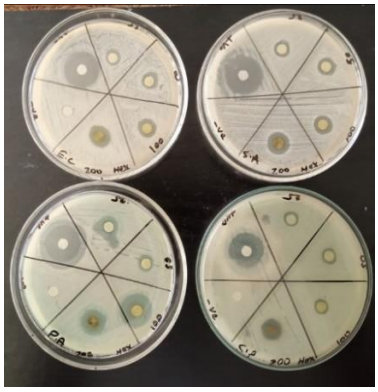
Ketoconazole	30	-	-	-	-	20.00±1.00
DMSO	1ml	0	0	0	0	0



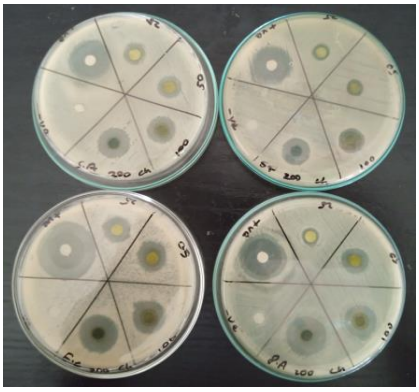
Distilled water



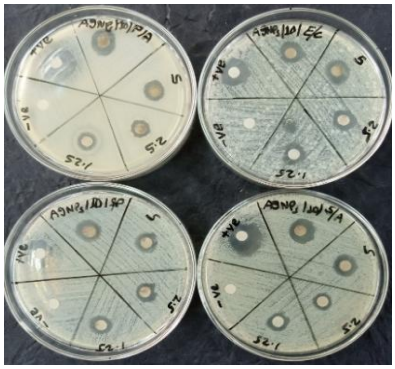
Methanol



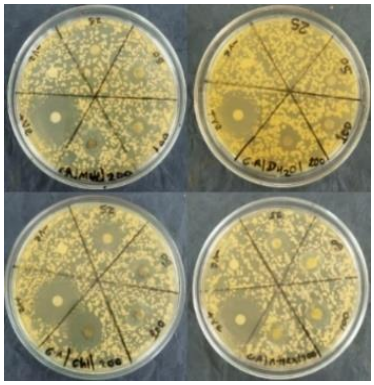
N-hexane



Chloroform



Silver nanoparticle against all bacteria strains



Antifungal activity on all extracts

AgNPs against *Candida albicans*

Figure 9. Antimicrobial activities of leaf extracts and green silver nanoparticles

4.5.1. Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was studied using different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml for leaf extracts, and 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.625 mg/ml for green synthesis silver nanoparticle against four bacteria strains and one fungus strain. The MIC value of the leaf extracts and the green synthesis silver nanoparticles determined the lowest concentration that inhibited bacterial and fungus growth after 24 hr for bacteria and 48 hr for fungus incubation at 37 ^{0}c. The results are shown in Table 7&9 below.

Table 7. The minimum inhibitory concentration (MIC) of leaf extracts of *Nicotiana glauca*

Plant 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

4.5.2. 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 Table 8&9.

Table 8. The MBC and MFC of all 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

Table 9. The MIC, MBC, and MFC for green synthesis silver nanoparticles

	Escherichia coli		*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Streptococcus pyogenes*		*Candida albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
AgNPs	1.25	10	1.25	5	2.5	5	2.5	5	0.625	5

5. DISCUSSIONS

When compared with distilled water, chloroform, and n-hexane, methanol produced the highest yield. When compared to the non-polar solvent, the polar solvent produced superior results. When compared to n-hexane, chloroform produced better outcomes. According to (Mohamed *et al*., 2022), methanolic extracts have the best yield when compared to other solvents since methanol is an excellent solvent for extracting both polar and non-polar compounds; this study agrees with this conclusion.

The image of nanoparticles is agglomerated, and the aggregation may be related to the secondary metabolites or salt concentration (Jemal *et al*., 2017). There may be some sort of bonding or interaction between them, which could affect their properties (Khairunnisa *et al*., 2021). 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.

Phytochemicals found in medicinal plants exhibit antimicrobial activity, anti-inflammatory activity, anticancer activity, antiallergic activity, antiviral activity, antimalarial activity, and other properties (Ali Alghamdi, 2021). Previous studies found polyphenols, flavonoids, tannins, coumarins, terpenes, steroids, alkaloids, and quinones in *Nicotiana glauca* leaf extracts. Dichloromethane extract contained alkaloids and terpenes, while ethyl acetate and n-butanol extract contained flavonoids and tannins. Saponins, on the other hand, were absent in all three solvent extracts (Trifa *et al*., 2020).

According to the phytochemical test results of (Najah *et al*., 2015), the aqueous extract of *Nicotiana glauca* contains flavonoids and alkaloids but no tannins, saponins, phenols, or glycosides. Saponins are lacking in chloroform extract, although glycosides are present. Flavonoids were not present in the n-hexane extract. The current findings disagree with some of the previous findings, since alkaloids, tannins, phenols, glycosides, and saponins were found in the aqueous extract. Flavonoids were also absent. Saponins were detected in chloroform extract, but glycosides were absent. Flavonoids were found in the n-hexane extract. The difference could be due to environmental factors such as mineral insufficiency, drought, cold, and disease (Hassan *et al*., 2014).

The antioxidant activity of the sample was measured in the current study using the DPPH scavenging assay at various concentrations. Ascorbic acid, silver nanoparticles, methanol, chloroform, and n-hexane exhibited (94.22%, 86.11%, 70.67%, 61.62%, and 58.34%) scavenging activities in 250 µg/ml concentrations, respectively. The lowest Ic50 value demonstrated the highest antioxidant activity. When the Ic50 values of the samples are compared to ascorbic acid 24 µg/ml, silver nanoparticles had the highest antioxidant activity 78 µg/ml. Methanol has an Ic50 value of 170 µg/ml from leaf extracts, chloroform has a value of 197 µg/ml, and n-hexane has a value of 202 µg/ml. The current investigation supports previous findings that methanol has high antioxidant activity when compared to other extracts (Gutiérrez *et al*., 2014).

The polar solvent extract was effective against gram-negative bacteria. The antimicrobial activity of *Nicotiana glauca* leaf extracts and silver nanoparticles ranged from 7.33±0.58 mm to 16.33±1.16 mm. Methanol extract had shown 15.33±1.16 mm while distilled water had shown 15.00±1.73 mm against *Escherichia coli*. While the chloroform extract was effective against *Streptococcus pyogenes* 15.00±1.00 mm, the n-hexane extract was more effective against *Staphylococcus aureus* 15.00±1.00 mm. The current work accords with (Gopalakrishnan *et al*., 2015) that silver nanoparticles have outstanding antibacterial and antifungal activity. The previous study found that silver nanoparticles have higher activity in gram-positive bacteria than in gram-negative bacteria (Sarsar *et al*., 2014), but the current study contradicts this finding. The n-hexane extracts had shown 13.00±1.00 mm and silver nanoparticles had shown 15.00±1.00 mm an inhibition zone against *Candida albicans*. Both n-hexane and silver nanoparticles showed better activity against *Candida albicans* than others. According to previous research (Ali Alghamdi, 2021), polar solvent extracts such as methanol and distilled water have higher activity than nonpolar solvents.

According to the findings, 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 MIC of the methanolic extract against *Candida albicans* was 12.5 mg/ml. The MIC in distilled water extract was 50 mg/ml for gram-negative bacteria, 25 mg/ml for gram-positive bacteria, and 25 mg/ml for *Candida albicans*. The MIC of the chloroform extract was 25 mg/ml in all bacterial strains and 12.5 mg/ml in *Candida albicans*. The MIC of the n-hexane extract was 25 mg/ml for *Escherichia coli* and 50 mg/ml for *Pseudomonas aeruginosa*, *Streptococcus*

pyogenes, *Staphylococcus aureus*, and *Candida albicans*. The MIC of silver nanoparticles in *Escherichia coli* and *Pseudomonas aeruginosa* was 1.25 mg/ml, and 2.5 mg/ml in *Streptococcus aureus* and *Staphylococcus pyogenes*. In *Candida albicans*, the MIC of silver nanoparticles was determined to be 0.625 mg/ml. The previous study (Mohamed *et al*., 2022) found that 4 µg/ml of *Nicotiana glauca* extract can inhibit the growth of *staphylococcus aureus* and 64 µg/ml of plant extract can prevent the growth of *Escherichia coli*; however, this study contradicts the current study.

MBC was found in the methanol extract at the maximum concentration of 100 mg/ml against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. The MBC and MFC concentrations in *Pseudomonas aeruginosa* and *Candida albicans* were 50 mg/ml, respectively. The MBC was found to be effective at 50 mg/ml against *Staphylococcus aureus* and 100 mg/ml against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* in distilled water extract. MFC was 100 mg/ml against *Candida albicans*. The MBC was effective against all bacterial strains in n-hexane extract at 100 mg/ml. The MFC was also detected at 100 mg/ml. The MBC was found in the chloroform extract at 100 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa* and 50 mg/ml against *Streptococcus pyogenes* and *Staphylococcus aureus*. N-hexane has an MFC of 50 mg/ml. MBC was detected in *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* at 5 mg/ml and 10 mg/ml in *Escherichia coli*. The MFC was shown against *Candida albicans* at 5 mg/ml.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The leave powder *Nicotiana glauca* was macerated in methanol, distilled water, chloroform, and n-hexane were analyzed for phytochemical presence was concluded that the presence of alkaloids, saponins, flavonoids, steroids, terpenoids, phenolics, tannins, and glycosides. The polar solvents contain more compounds than nonpolar solvents. The antioxidant activities of *Nicotiana glauca* leaf extract and green synthesis silver nanoparticles indicate 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 activity than plant extracts. Finally, the plant *Nicotiana glauca* has a strong potential for antioxidant and antimicrobial activity.

6.2. Recommendations

Further studies are necessary for screening the phytochemicals that are present in the plant *Nicotiana glauca* because it is an essential component of antimicrobial and antioxidant activity and for other biological activities. Secondary metabolites isolation and characterization of plant *Nicotiana glauca* should be done. The antimicrobial activity studies should be done by using different concentrations. Antimicrobial activities should be done against pathogenic microorganisms. Plant *Nicotiana glauca* has a capacity for different biological activities so further studies on the biological activities of this plant should be done.

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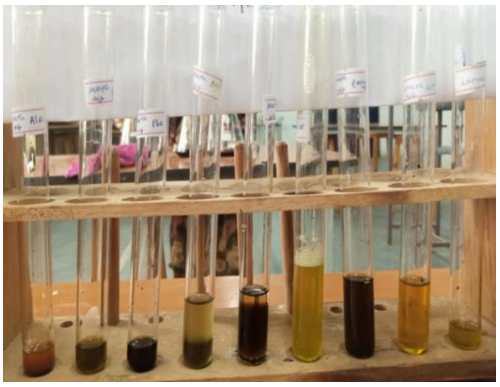
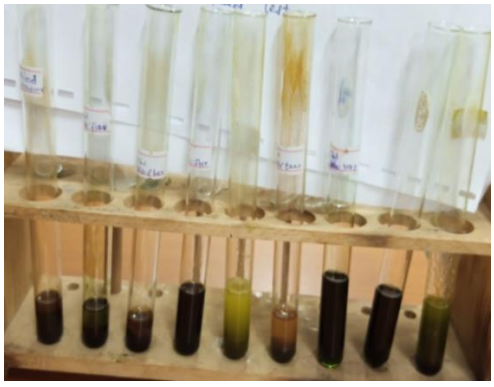
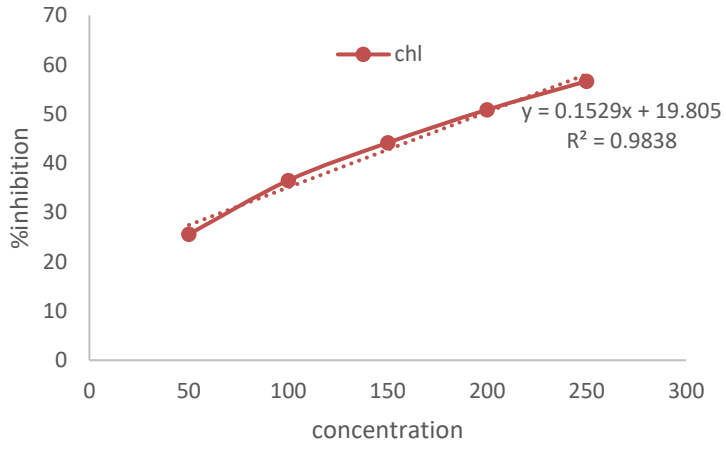
8. APPENDICES

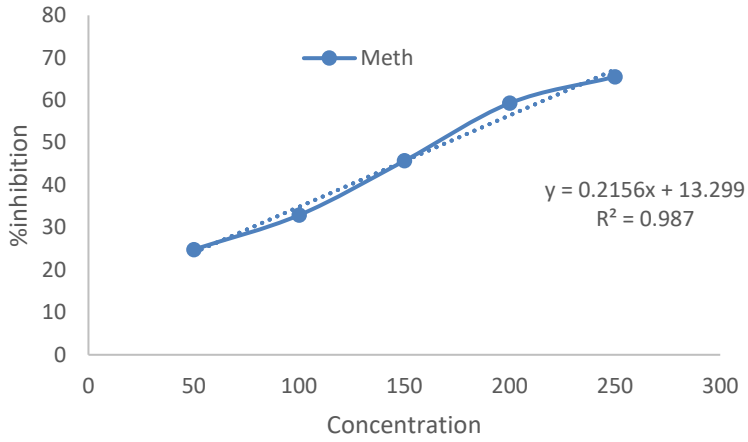
Appendix 1: The figure showed the results of phytochemical tests: A. methanol extract, B. distilled water, C. n-hexane extract, and D. chloroform extract

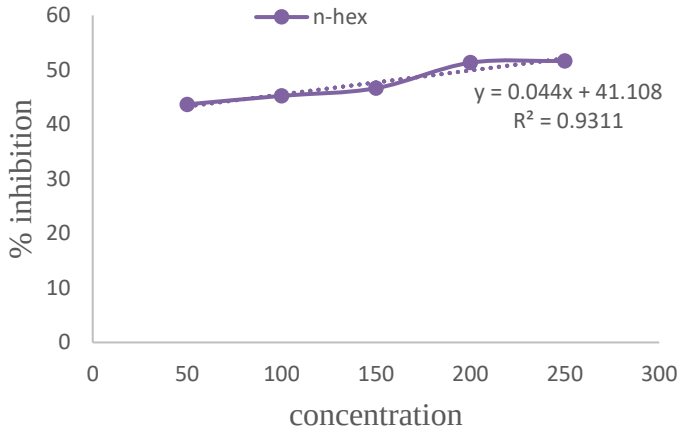
A.  B. 

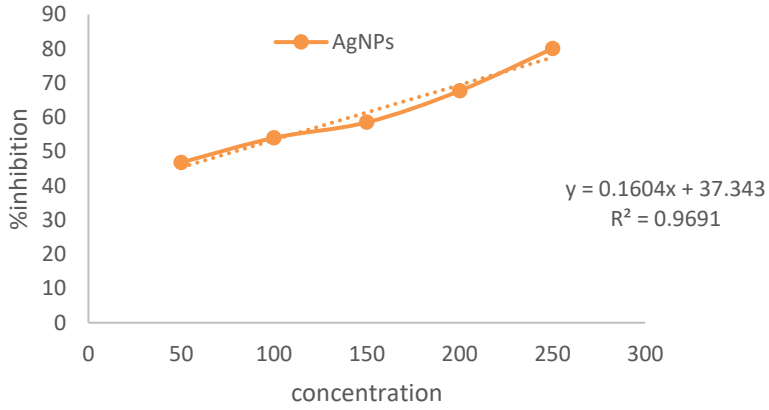
C.  D. 

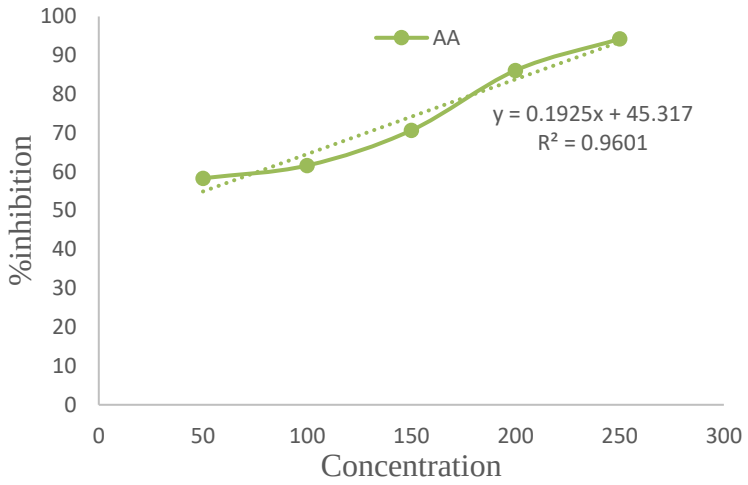
Appendix 2: Graph showed the Ic50 values of ascorbic acid, extracts, and green synthesis silver nanoparticles




Meth
%inhibition
Concentration
y = 0.2156x + 13.299
R² = 0.987
0
10
20
30
40
50
60
70
80
0
50
100
150
200
250
300


n-hex
% inhibition
concentration
y = 0.044x + 41.108
R² = 0.9311
0
10
20
30
40
50
60
0
50
100
150
200
250
300


AgNPs
%inhibition
concentration
y = 0.1604x + 37.343
R² = 0.9691
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250
300



Appendix 5: Plant identification

Collected by Tsion Guta

Identified by Melaku Wendaferash AAU.

No	Code No.	Local Name	Family	scientific Name
1	TG001	Yeareb Kitel	Solanaceae	Nicotiana glauca R. Grah