Phytochemical Analysis and Evaluation of *Invitro* Antimicrobial and Antioxidant Activities of the Leaf Extracts and Leaf Extract Mediated Silver nano Particles of *Verbascum sinaiticum* Benth

Konjit Habtamu

MSc Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

June, 2023

Adama, Ethiopia

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Advisor: Dr. Kero Jemal

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Declaration

I declare that this thesis entitled **"Phytochemical Analysis and Evaluation of *In-vitro* Antimicrobial and Antioxidant Activities of the Leaf Extracts and Leaf extract Mediated Silver nano Particles of *Verbascum sinaiticum* Benth,"** was my own work and hadn't been submitted to any university for similar purpose. The references used in this thesis are duly recognized by proper citations.

_____ _____ _____

Name of student Signature Date

Advisor Recommendation

I, the advisor of this MSc thesis, hereby certify that I have read the revised version of the thesis entitled "**Phytochemical Analysis, Evaluation of *In Vitro* Antimicrobial and Antioxidant Activities of the Leaf Extract and Leaf extract Derived Silver nano particles of *Verbascum sinaiticum* Benth**," prepared under my guidance by Konjit Habtamu. And submitted in partial fulfilment of the requirements for the Masters of science in Biotechnology.

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

Advisor	Signature	Date
_____	_____	_____

Advisors Approval Sheet

This is to certify that the thesis entitled "**Phytochemical Analysis and Evaluation of *In vitro* Antimicrobial and Antioxidant Activities of the Leaf Extracts and leaf extract derived Silver nano particles of *Verbascum sinaiticum* Benth**" is submitted in partial fulfillment of a degree Master Science in Biotechnology, to the graduate program of applied Biology and developed by Konjit Habtamu. 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 proposal by Konjit Habtamu have read and evaluated, Thesis entitled **Phytochemical Analysis, Evaluation of *Invitro* Antimicrobial and Antioxidant Activities of the Leaf Extracts and Leaf extract Mediated Silver nanoparticles of *Verbascum sinaiticum* Benth** and examined the candidate during the open defense. Therefore, this is to certify that the MSc thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Biotechnology.

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The final approval and acceptance of the thesis proposal are dependent or contingent upon the submission of its final copy to the office of Postgraduate studies through the Department of the graduate council and the school graduate committee.

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Acknowledgement

Frist and for most I would like to thanks the Almighty God who gave me health and strength to start and finalize this study. Next, I would like to express my deepest gratitude and respect to my advisor Dr. Kero Jemal for his comments, advices, chemical support and helpful guidance throughout my thesis.

I would like to thank to Mr Lata Guta for his assistance by giving the chemicals, apparatus and guidance during laboratory activity of my thesis. I would also like to thank Mr Mukter for his aid in allowing/ permit me to use UV spectrophotometer and technically support by devoting his time during laboratory activity.

I would like thank to Biology department of Adama Science and Technology University for instrumental and chemical supports. Many thanks also go to Ministry of Education and Mettu University for sponsoring my MSc study.

I would like to express grateful thanks to my beloved family/brother/ and friend for their patience and giving moral support for me in a lot to complete this thesis successfully.

Table of Contents

Contents **Page**

List of figures

Figures **Page**

List of Tables

Tables **Page**

List of Acronyms and Abbreviations

AgNps……Silver nano particles

DMSO……. Dimethyl sulfoxide

DNA……Deoxyribonucleic acid

DPPH…….2,2 diphenyl-1-picrylhydrazyl

FRAS…… Ferric reducing antioxidant power

JCPDS……. Joint committee on powder diffraction standards

MBC……Minimum bactericidal concentration

MDR……multi-drug resistant

MFC……Minimum fungicidal concentration

MHA…….....Muller Hinton agar

MIC……...Minimum inhibitory concentration

ORAS……. Oxygen radical absorption capacity

OH……Hydroxyl

ROS……Reactive oxygen species

SDA…….....Sabroud Dextrose agar

SEM……Scanning electron microscopy

SPSS……Statistical package of social science

TM……Traditional medicine

UV……Ultra violet

WHO……World health organization

XRD…….X-ray diffraction

Abstract

Verbascum sinaiticum (Mullein) is one of the medicinal plants, traditionally used to cure various human diseases including skin disorders, fungal infection, stomachache, elephantiasis, wound and several animal ailments. Phytochemical investigation and evaluation of biological properties of traditionally used medicinal plants such as Verbascum sinaiticum in Ethiopia haven't been widely studied. The aim of this study was to investigate the phytochemical constituent, evaluation of antimicrobial and antioxidant activities of leaf extracts and leaf extract-mediated green synthesized silver nanoparticles of V. sinaiticum. Leaves powder of V. sinaiticum was macerated with methanol, distilled water, acetone, chloroform and n-hexane solvents separately for extraction. The qualitative phytochemical analysis was performed by using standard methods and antimicrobial activity of crude extracts and silver nanoparticles were done by Disc diffusion assay against selected bacterial and fungal species. The Minimum inhibitory concentrations of crude extracts and silver nano particles were determined by broth dilution methods. The antioxidant activities were evaluated by DPPH assay. Silver nanoparticles were synthesized by mixing silver nanoparticles and plant leaf extract. Synthesized silver nanoparticles were characterized by XRD and SEM. The results of phytochemical compositions showed the presence of alkaloids, flavonoids, saponins, steroids, phenols, tannins, glycosides and terpenoids in different crude extracts of V. sinaiticum leaf. The XRD pattern confirmed the green synthesized silver nanoparticles were in nano crystalline nature with face cubic centered structure. SEM analysis indicated highly agglomeration of silver nanoparticles with spherical shape. Antimicrobial activity of the extract and silver nanoparticles showed maximum zone of inhibition against S. aureus (17±1.0mm), C. albican (13.0±1.0mm) and E. coli(16.0±1.0mm), C. albican (15.0±1.0mm) respectively. Methanol and chloroform extract exhibited significantly($p<0.05$) a high antimicrobial activity. Whereas acetone and distilled water extract shown moderate antimicrobial activity. The minimum inhibitory concentrations values of the leaf crude extracts and silver nanoparticles were range from 12.5 to 50mg/ml and 0.625 to 1.25mg/ml respectively. The minimum bactericidal and fungicidal concentration values ranged from 50 to 100mg/ml and 5 to 10mg/ml for crude extracts and silver nanoparticles respectively. Methanol extract and silver nanoparticles exhibited very high radical scavenging activity with IC_{50} values 49.42 and 18.35µg/ml respectively. Whereas acetone crude extract revealed low radical scavenging activity with high IC_{50} values (172.62 µg/ml). This result suggests crude extracts and Leaf extract mediated AgNps of V. sinaiticum exhibited both antimicrobial and antioxidant activity. Various therapeutic agents present in the leaf extracts of this plant are promises this plant to be studied further.

Keywords: Medicinal plant, *V. sinaiticum*, phytochemicals, antimicrobial, antioxidant, silver nano particles

1. Background

1.1. Introduction

Medicinal plants play a crucial role in the development and advancement of modern studies by serving as a starting point for the development of novel drugs and medicinal purposes. According to World Health Organization, traditional medicine (TM) is the total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures and nations which are handed from generation to generation whether in writing or orally without documentation (Eshete & Molla, 2021; Talema, 2020). According to the World Health Organization, 21,000 plants species are used as medicinal plants (Hoareau & DaSilva, 1999). Recently, WHO estimated that 80% of the world's population relies on these plants to meet their primary health problem or for their primary health care needs (Abebe & Mekonnen, 2016).

Ethiopia has a wealth of biodiversity which consists of approximately 6500 to 7000 plant species among them 21% are endemic(Yordanos & Germame, 2021). Like other developing countries, Ethiopians use traditional medicines to meet their primary health care both in rural and urban areas (Bitew *et al.*, 2019). Medicinal plants have a vital role in traditional medicine to meet the health care system for human and animal ailments in various developing countries including Ethiopia(Tesfahuneygn & Gebreegziabher, 2019).

Various studies showed that 80% of the human population and 90% of livestock in Ethiopia depend on traditional plant-based medicine (Regassa, 2013; Tesfahuneygn & Gebreegziabher, 2019). This is due to the cultural acceptability of healers, difficulties in obtaining modern health facilities and the low cost of traditional medicine (Nguta *et al*., 2010) The use of medicinal plants, is not only for curing diseases but also essential for the protection and promotion of human physical, spiritual, social and mental (Agidew, 2022). Recently, different parts of medicinal plants have been used to cure specific ailments. For centuries, mankind uses many species of medicinal plants to treat diseases and prevent infections. This is, because of their ability to produce secondary metabolites or bioactive compounds such as flavonoids, alkaloids,

essential oils, phenols and etc. (Equar *et al*., 2018; Shifa *et al*., 2021). These medicinal plant secondary metabolites have antimicrobial, antioxidant, anti-inflammatory, anthelmintic, anticancer, wound healing and insecticidal activities (Sisay *et al*., 2019; Dini *et al*., 2022; Pareek *et al*., 2023).

Plants have variations in their phytochemical constituents and biological activities. Their phytochemical constituents and biological activity variations have been determined by plant quality or features and pre-treatment factors (Renard *et al.,* 2017; Ali & Endalew, 2021) . These factors rely on geographical location, plant parts used, climatic conditions, collection period, drying methods, the solvent used for the extraction process and drying method (Li *et al*., 2012; Renard *et al*., 2017; Krakowska-Sieprawska *et al*., 2022). Determination of the pharmacological and phytochemical activity of several plants that were similarly used in different parts of the world leads to the innovation of novel structures for the manufacture of new drugs. For example, studies in China showed that 104 new drugs developed over 37 years, used 60 medicinal plants in the traditional medicine system (Geyid *et al*., 2005).

The accessibility and affordability of many currently prescribed antibiotics worldwide have been compromised through the influence of multi-drug-resistant pathogens which reduced their effectiveness and increases healthcare costs, morbidity and mortality. According to several reports, the total economic cost of antibiotic resistance was estimated at around $20 billion to 35 billion dollars in direct health care and productivity within a year (Kebede *et al*., 2021).In developing countries or low-income countries, this situation is further difficult because of the absence of laboratory diagnostics, effective surveillance systems and services of appropriate antimicrobials. If there is no alternative action to obtain new drugs, on the world the number of mortalities will rise to 10,000,000 and costs up to $100 trillion by the year 2050 (Bakal *et a*l., 2017).

To overcome these challenges antibiotics derived from the natural bioactive compound are the best alternatives (Equar *et al.,* 2018). Medicinal plants are highly targeted to search for new antimicrobial agents (Sisay *et al*., 2019). Various studies have shown that medicinal plants

contain bioactive compounds such as coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes which serve as a turning point for the synthesis of antibiotics (Parvin *et al.*, 2014; Abebe & Mekonnen, 2016).

Free radicals are unstable atoms/molecules with unpaired electrons produced during normal cellular processes. These un-paired electrons have the capability/potential to become stable by pairing with other biomolecules of human cells including protein, carbohydrate, DNA and lipids which reason to damage macromolecules (Islam *et al*., 2013). This damaging leads to increasing oxidative stress in human various disorders like cancer, diabetes, neuro-degenerative, cardiovascular and aging process (Phaniendra & Babu, 2015). The dietary consumption of the source of antioxidants is known to reduce the risk of diseases caused by these free radicals (Pham-huy *et al*., 2008). Since antioxidants are the substance that can able to prevent free radicals from our body by scavenging them by donating electrons. Historically, fruits, cereals and vegetables have been recognized as sources of antioxidants (radical scavengers) because of the presence of numerous phytochemicals like carotenoids, vitamin C, Vitamin E and polyphenols (Lü *et al*., 2010; Bansal *et al*., 2013). Plant-derived bioactive compounds have shown antioxidant activities, which is safer as well as healthier than synthetic antioxidant (Gulcin, 2020).

Nanotechnology is now creating/emerging science which is a growing sense of excitement in life science especially in biotechnology and biomedical devices (Ingle *et al*., 2017). It deals with nanoparticles having small sizes ranging from 1nm to 100nm. Nano particles are perceived great interest of studies because of their small size and large surface-to-volume ratio than the same bulk. Green nanotechnology is one of the leading technologies synthesis of metallic nanoparticles eco-friendly by using biological materials including plants, algae, fungi and bacteria (Chandan *et al*., 2021).On the other hand, nanoparticles can able to synthesized by a physical, chemical and hybrid system. Nevertheless, these methods are time-consuming process, expensive and nanoparticles synthesized by them are produced by toxic and hazardous materials that are toxic to the environment (Patra & Baek, 2014). Green synthesis of nanoparticles has received/ influenced the interest/ attention of the scientific community due to its less cost,

effectiveness, eco-friendly and easy process (Oves *et al*., 2018). Nanoparticles have shown novel and improved properties that rely on their specific characteristics such as size, distribution and morphology. Among other metal nanoparticles, AgNps are the most widely studied because of their indispensable application in various fields like biomedical, catalysis, electronics, antimicrobial and several industries including food packing, textiles, leather, paint and paper (Sathiyavimal *et al*., 2018; Jacob *et al*., 2019).

Historically for several centuries, silver has been known as a disinfecting agent as well as employed in traditional medicine and culinary items/cooking. Silver nanoparticles have the potential to against bacteria, fungi, viruses, cancer, and other eukaryotic microorganisms and also haven't any side effects/non-toxic to humans. In the plant-based synthesis of silver nanoparticles methods, the bioactive compounds include terpenoids, alkaloids, tannins, phenols, flavones, polysaccharides and proteins that existed in the medicinal plant have the capability to produce AgNps by serving as stabilizing and capping agent to reduce silver ion into AgNps (Oves *et al*., 2018).

Verbascum sinaiticum is one of the medicinal plants used traditionally in Ethiopia to treat different diseases. It is a member of the Scrophulariaceae family within the *Verbascum* genus which is the dominant genus of the family which consists of about 360 species among a large number of species. According to several studies, the Verbascum genus or the species of this genus consists of numerous bioactive compounds including flavonoids, phenolic acids, sterols, triterpenes, saponins, polysaccharides, alkaloids and iridoid glycosides with different biological activity (Mahmoud et al., 2007). This plant is used traditionally to cure diarrhea, hemorrhage, measles, anthrax, wound, superficial fungal infection, elephantiasis, anthrax, syphilis, gonorrhea, mental illness, and etc. (Lulseged *et al*., 2022; Tadeg *et al*., 2005; Yeabyo *et al*., 2018).

Therefore, the aim of this study was to screen phytochemicals present in its leaf extracts, evaluate the antimicrobial and antioxidant activities of leaf extracts and aqueous leaf extract derived green synthesized silver nanoparticles of *Verbascum sinaiticum*.

1.2. Statement of the problem

Medicinal plants play an indispensable role in the daily lives of people living in developing countries of Asia and Africa, including Ethiopia. Medicinal plants are not only used as substitutes for modern medical treatments, which are often inadequately available but also improve the health of the local people (Agidew, 2022). Globally, around 75–90% of the rural population of developing countries depend on traditional medicines as their primary healthcare system (Chekole, 2017). This is not only due to their low income in which people are unable to buy expensive modern synthetic drugs. However, traditional practices of medicinal plants have been more culturally acceptable and fit psychological needs than modern medicine (Fassil, 2001).

Several studies have been reported on ethnobotanical and ethnopharmacological evidence of the number of Ethiopian medicinal plants which are traditionally used to treat a variety of diseases such as skin disorders, cancer, stomachache, headache, evil eye, leprosy, fungal infection, diarrhea, eczema, and wound (Talema, 2020; Kebede *et al*., 2021; Shifa *et al*., 2021). However, there is limited development of medical products from these plants and the practice of indigenous knowledge become declined or lost due to the migration of the population from rural to urban areas, loss of natural habitat, and industrialization (Eshete & Molla, 2021).

Due to an increase in the resistance of different pathogens, such as bacteria, fungi, and viruses, to antibiotics as well as a dearth of medications used to treat infectious diseases, controlling infectious diseases is becoming more and more challenging. Finding alternative antimicrobial compounds from plants is one approach to solving this issue. In an effort to combat antibiotic-resistant diseases, more research is being done now to explore new bioactive compounds from plant origin (Subramani *et al*., 2017). There are about 200,000 bioactive compounds derived from plants, yet this still represents only a fraction of the compounds produced by plant species that are native to the Earth (Efferth & Koch, 2011).

Another problem related to human health is the presence of reactive oxygen species /free radicals. Free radicals are atoms, molecules, or ions with unpaired electrons which are derived

from elements like oxygen, nitrogen and sulfur. For instance: free radicals which are originated from oxygen known as Reactive oxygen species (ROS) includes, including hydroxyl, superoxide, peroxyl, alkoxyl, and nitric oxide. They are highly unstable and active in chemical reactions with other molecules. Free radicals produced in our bodies create abnormal physiological conditions that cause oxidative damage to cells through lipids, proteins, and nucleic acid biomolecule degradation. As a result, this oxidative damage leads to various pathologies such as arthritis, cancer, age-related degenerative brain disorders, and coronary diseases. This damage is repaired by antioxidants which convert the generated free radicals into less harmful molecules. Antioxidants have shown a defense action system through several mechanisms such as direct reaction with free radicals, chelation transition metals, activation or stimulation of antioxidant enzymes and reduction of peroxides (Parr & Bolwell, 2000).

Several synthetic antioxidants are commercially available, to be used in the food industry as food additives and pharmacological action, however; Recently they have been reported to be toxic (Liu *et al*., 2011). Some of the Synthetic antioxidants have been responsible for liver damage and Carcinogenesis, as well as causing damage to the helical structure of DNA (Dolatabadi & Kashanian, 2010). Due to these negative health effects(drawbacks of synthetic antioxidants), they need to be substituted with natural antioxidants that occur in medicinal plants including; phenolics, flavonoids, proanthocyanidins and etc. (Anraku *et al*., 2018). Bioactive compounds derived from plants have antioxidant properties and the capability to prevent oxidative stress (Aiyegoro & Okoh, 2009).

The phytochemical constituents of the most medicinal plants used traditionally in Ethiopia are not studied extensively. This leads to more traditionally used medicinal plants in Ethiopia being unrecognized by an international scientific organization, and their antimicrobial and antioxidant activities are not well studied. Phytochemical screening of medicinal plants in Ethiopia is limited, so further study is needed for investigation and characterization of the properties of the medicinal plants in all parts of Ethiopia from the whole parts of plants such as roots, leaves, seed, stem, etc. (Kebede *et a*l., 2021; Agidew, 2022). In order to encourage the use of medicinal

plants as antimicrobials and antioxidants, the evaluation of their phytochemical composition and biological activity is very important.

The use silver nanoparticle to solve problem is currently increasing. Several decades ago, silver and its various salts have been served as antimicrobial agents in primary health care and wound treatment (Saravanan *et al*., 2018; Shanmuganathan *et al.,* 2018). The medicinal values of silver have been recognized almost for 2000 years. Recently in the nineteen-century silver and silver-derived compounds have been used in antimicrobial applications (Rakib-Uz-Zaman *et al*., 2021). However, the employment of silver and its compound were gradually reduced due to the development of novel new antibiotics (Deljou & Goudarzi, 2016). In recent years they have been a growing interest in nanotechnology. Because the application of nanoparticles in medicine, industries, pharmaceutical and agricultural fields has been highly recognized (Hussein *et al*., 2019).

The development of drug-resistant pathogenic microbes has become a major challenge in human health (Morens & Fauci, 2013; Tong *et al*., 2018). Because the emergence of currently available antibiotics resistance and their toxicity limit the utilization of antibiotics in health areas/ clinics to treat human pathogenic diseases. To overcome this drawback, green nanotechnology promises an alternative method to cure these microbial infections through synthesizing silver nanoparticles which have potent antimicrobial activity with their effectiveness than standard antibiotics used in clinical areas (Loo *et al*., 2018; Kotcherlakota *et al*., 2019). On the other hand, in order to against MDR microbes synthesizing/developing new antibiotics is time expensive and consuming-process. Due to the fact that Nanotechnologies and silver nanoparticles are the best alternative sources to resolve this problem of antibiotics resistance (Desai & Kelmani, 2016) Recently, numerous studies have demonstrated the potential AgNps as the best choice antimicrobial agents (Liu *et al*., 2010; Das *et al*., 2020). Additionally, the strong free radical scavenging activity of these nanoparticles has been investigated by several studies, because of the presence of various bioactive compounds on the surface of plant extract-mediated synthesized AgNps (Ahmed *et al*., 2019; Nagaich *et al*., 2016).

Biosynthesis of AgNps is a more advantageous approach than other methods of AgNps synthesis due to its easy synthesis route, eco-friendly process, low cost, non-toxic, excellent performance, and involvement of green synthesized AgNps in the medical sector without any side effects. This biological method has vastly relied on medicinal plant or plant-based extracts which is free from toxic chemical completely, fast process, nonpathogenic, easy to handle and safe, environmentally suitable and have lower cultivation cost when compared with other biological approaches (microbes-based green synthesized) (Ismail *et al*., 2016; Beyene *et al*., 2017). The presence of secondary metabolites in medicinal plants contributes to plant extract to reduce Ag ion into AgNps which has a significant role in the medical and pharmaceutical area because of its antimicrobial, anti-inflammatory, antioxidant and anti-cancer properties (Kathiravan *et al*., 2014; Pugazhendhi *et al*., 2018). This study produces silver nanoparticles for the first time from leaf extract of *Verbascum sinaiticum* and evaluates its antimicrobial and antioxidant activity on the selected human pathogenic strains.

Therefore, various studies must be carried out in different parts of Ethiopia to identify the phytochemical constituents and their biological activity by using different methods and solvents for the extraction of essential bioactive compounds from these medicinal plants for re-assuring their importance through scientific investigation. Due to the fact that the bioactive compound constituents of plants and their activities are influenced by environmental factors, geographical conditions, the solvent used and other factors (Ali & Endalew, 2021).

It is important to screen the phytochemical constituents of medicinal plants and test for the antimicrobial and antioxidant activities of their extracts. One of the traditionally used medicinal plants in Ethiopia is *Verbascum sinaiticum.* Its phytochemical constituents, antioxidant and antimicrobial activity haven't been studied widely and are unknown in detail. Finally, the aim of this study was to investigate preliminary phytochemical constituents and evaluate the antimicrobial and antioxidant activities of *Verbascum sinaiticum* leaf extracts. Additionally, the aim of this study was to green synthesis of silver nanoparticles from the aqueous leaf extract of this plant and examine its antimicrobial and antioxidant activity.

1.3. Research questions

This research was answering the following questions:

1) What are the phytochemicals present in different extracts of leaves of *Verbascum sinaiticum*?

2) Do the leaf extracts of *Verbascum sinaiticum* have antimicrobial activity?

3) Do the leaf extracts of *Verbascum sinaiticum* have antioxidant activity?

4) Do the aqueous leaf extract of *Verbascum sinaiticum* can synthesize silver nanoparticles?

5) Do the green synthesized AgNps have antimicrobial and antioxidant activity than leaf crude extracts of *Verbascum sinaiticum*?

1.4. Objective of the study

1.4.1. General objective

The main objective of this study was to investigate the phytochemical constituent of *Verbascum sinaiticum* leaf extracts and evaluation of antimicrobial and antioxidant activities of leaf extracts and leaf extract mediated synthesized AgNps.

1.4.1.1. Specific objectives

The specific objectives of this study were:

- ✓ To determine the phytochemical constituents of different solvent extracts of the leaf of *Verbascum sinaiticum*
- ✓ To evaluate the antimicrobial activities of the different solvents of leaf extracts of *Verbascum sinaiticum*
- ✓ To evaluate the antioxidant activities of different solvents of leaf extracts of *Verbascum sinaiticum*
- ✓ To synthesize silver nanoparticles by using aqueous leaf extract of *Verbascum sinaiticum*

- ✓ To evaluate the antimicrobial and antioxidant activities of green synthesized silver nanoparticles using aqueous leaf extract of *Verbascum sinaiticum*

1.5. Scope of the study

The study was conducted at Adama Science and Technology University in the Laboratory of applied biology, school of applied sciences. The plant material was collected only from the Wachale district North Shewa Oromia regional state of Ethiopia. The study was focused on phytochemical investigation or screening, evaluation of the antioxidant and antimicrobial activities of the *Verbascum sinaiticum* leaf extracts. Additionally, this study also covered the green synthesis of silver nanoparticles from the aqueous leaf extract of this plant and examination of its antimicrobial and antioxidant activity and characterization of the synthesized silver nanoparticles by using SEM and X-RD. The antimicrobial test was carried out only on the selected strain of bacterial and fungal species which cause various illnesses in human beings. The *in-vitro* antioxidant activity of the leaf extract was evaluated by the DPPH method.

1.6. Significance of the study

The finding of this study will help people in the study area to be aware of problems associated with the medicinal plant, give attention to their use as well as conservation and ensure the importance of this medicinal plant through scientific investigation or may give a scientific base for medicinal use of leaf part of *V. sinaiticum*. This research is adding some knowledge about *Verbascum sinaiticum* by extracting various phytochemicals from the leaf part of this plant by using different types of solvents as well as providing its effectiveness in antimicrobial and antioxidant activities. Furthermore, this study will providing scientific-based knowledge or add knowledge to the scientific community about green synthesized AgNps from *Verbascum sinaiticum* and its antimicrobial activity. Finally, this study also served as a standing stone or input for further study on the leaf part of this plant.

2. Literature Review

2.1. Medicinal plants

Medicinal plants are any plants that in one or more of its organs contain substances that can be used for therapeutic purposes or precursors of the synthesizing of valuable drugs. These substances are important in preventing and treating various ailments and diseases which are considered to be beneficial in the health care system. They contain promising molecules used by the population of the world to meet their primary healthcare system (Nigussie *et a*l., 2021). These natural compounds are known as secondary metabolites which have no direct contribution to their growth, development, and metabolism (Equar *et al*., 2018). Ruther plays a role play in defense against plant enemies (biotic and abiotic stresses), and pollution, provides the ability to survive stress, UV rays and also contributes to the color, flavor, and aroma of the plant (Balamurugan & Velurajan, 2019). They are classified into three major groups includes phenolics (45%), terpenoids and steroids(27%), and alkaloids(18%)(Saxena *et al.,* 2013).

These compounds possess different biological properties within plants that are contributed to treating a wide spectrum of diseases including cancers, neurological disorders, chronic inflammation and lesions, particularly due to diabetes, cardiovascular diseases, and wounds (Górniak *et al*., 2019; Nigussie *et al.,* 2021). Medicinal plants have a vital role in antimicrobial activities. Medicinal plants have antimicrobial properties because of rich in various secondary metabolites, such as terpenoids, tannins, flavonoids, and alkaloids(Sisay et al., 2019).

In our body, various biochemical reactions can generate an unbalanced quantity of free radicals which results in oxidative damage of lipids, proteins, and other important biomolecules by creating abnormal physiological conditions. This oxidative damage was responsible for the development of several illnesses such as cancers, diabetes, cardiovascular disorder, neurodegeneration, aging, and others (Khanal *et al.,* 2020). Natural antioxidants have an indispensable role to repair the damage caused by the reactive oxygen species (ROS) by converting free radicals into harmful molecules by radical chain reaction. On the other hand,

Plant-derived natural compounds or bioactive compounds have a paramount role in mitigating oxidative stress due to their antioxidant activity (Ahmed *et al*., 2019; Mahmood *et al*., 2019).. Researchers are now devoted to the progress of innocent or harmless biologically active plant-derived compounds to employ for the development of novel drugs(Gaire & Subedi, 2011) The involvement of these drugs in medical practice has shown that they are relatively non-toxic, safe, and even free from serious side effects (Górniak *et a*l., 2019).

2.2. Phytochemical compounds in medicinal plants

Phytochemicals are bioactive compounds that are naturally active, occurring in plants and provide health benefits for the human being. They are protecting plants from damage or several environmental hazards and diseases and provide color, flavor as well as aroma to plants. To date, more than 4500 phytochemicals compounds are identified and 350 of them are studied in detail (Koche *et al*., 2016) They are divided into different classes based on their functional protective role and physical and chemical characteristics (Saranya & Divyabharathi, 2019). Different major classes of these compounds are described as follows:

2.2.1. Phenolics

Phenols sometimes referred to as phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group and widely distributed in the plant kingdom. Phenols are the largest group of secondary metabolites which are widely existed in plants. Phenols are a member of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group. There are three most important groups of dietary phenolics includes flavonoids, phenolic acids, and polyphenols. Phenolics are served as defense compounds and possess several properties beneficial to humans through defense against various diseases and their antioxidant properties provide a crucial role in preventing free radical-mediated diseases (Saxena *et al*., 2013).

2.2.2. Flavonoids

Flavonoids are also known as bioflavonoids polyphenolic antioxidant compounds that are existed everywhere in nature. A large number of Flavonoids have been recognized, in which many of them are found in vegetables, fruits, and beverages like tea, coffee, and fruit drinks. They have existed as glucosides, aglycones, and methylated derivatives. Flavonoids have

obtained various attention from scientists because of their wide biological and pharmacological activities such as cytotoxicity, antimicrobial, anti-inflammatory, enzyme inhibition, vascular activity, antitumor activities, etc.(Lin & Weng, 2006). However, the most important powerful properties of several classes of flavonoids are antioxidant activity. The potential of such type of activity of these compounds has relied upon their molecular structure, the position of the hydroxyl groups, and other attributed features in chemical structures.

2.2.3. Tannins

Tannins are toxins that have the potential to reduce the growth and survivorship of many herbivores when added to their diets. They also act as feeding repellents for a great diversity of animals. In addition, due to their properties of binding with salivary protein, enhance unpleasant, sharp, and astringent sensations within the mouth of the human and can activate herbivores' digestive enzymes by binding with protein (Chappell & Hahlbrock, 1984). They are classified into two major categories namely condensed and hydrolyzable tannins. Hydrolyzable tannins are heterogenous polymers containing phenolic acids, especially simple sugars, and gallic acid. While the condensed one is formed by the linkage of flavonoids unit and common constituents of woody plants that can be hydrolyzed to anthocyanidins through treatment with strong acids. Due to this referred to as proanthochocyanidins. Tannins are secondary metabolites compounds that have shown various biological activities such as antimicrobial activity, anti-inflammatory, antiseptic, antioxidant, against stomachache and diarrhea (Dolara *et al*., 2005).

2.2.4. Alkaloids

Alkaloids are a natural product and their name is derived from alkaline which describes a nitrogen-containing base. These compounds are naturally synthesized by animals, plants, bacteria, and fungi. However, alkaloids are primarily found in plants, especially in flowering plants, and metabolite by-products that are derived from amino acids (Naseem *et al*., 2014). In the early 19^{th} century among natural bioactive compounds isolated from medicinal plants, alkaloids were the dominant compound and were known as vegetable alkalis with a bitter taste(Saxena *et al*., 2013). Alkaloids have numerous biological and pharmacological activities including antimicrobial, insecticidal, antimalarial, antihypertensive, antiarrhythmic effects, and

anticancer action (Wink *et al*., 2004).

2.2.5. Saponins

Saponins are secondary metabolites widely distributed in plants. They are an important group of glycosides which are containing one or more sugar chains on a triterpene or steroid aglycone backbone also called a sapogenin. They form stable foam such as soap in an aqueous solution and their name is derived from the Latin word 'sopa', which means the plant consisting of the frothing agent when diluted in an aqueous solution (Saxena *et al.*, 2013). Saponins include various compounds with structural diversity which are reflected in their biological and physiochemical properties such as contributing to the number of traditional practices such as soaps, fish poison, pes, and molluscicides as well as industrial applications. In addition, plant extract containing saponins has served as surface active, detergents and foaming agents in the industry and is widely used in food. However, traditionally considered anti-nutritional and in some cases limited their use due to their bitter taste. Moreover, these compounds also have several health benefits because their structural variety act as an antioxidant to impair protein digestion and uptake of the vitamin and minerals in the gut, also as antiviral and fungal (Juang & Liang, 2020).

2.2.6. Terpenoids

Terpenoids are a class of natural products that have been derived from five-carbon isoprene units and classified into several classes according to the number of isoprene units (Langenheim, 1994). Terpenes are boundless in nature and mainly or especially distributed in plants as constituents of essential oil. These natural products have several commercial applications such as flavor and fragrances in foods and cosmetics. Various plants produce volatile terpenes in order to attract pollinators such as insects. Terpenoids have exhibited potential medicinal properties, such as antibacterial, antimalarial, antiviral, inhibition of cholesterol synthesis, anti-ulcer, anti-inflammatory, hepaticidal, anticarcinogenic and anti-cancer activities (Dudareva *et al*., 2004; Langenheim, 1994).

2.2.7. Steroids

Steroids are secondary metabolites with diverse structures and biological functions. They are organic compounds with four cyclohexane rings and are derived from cholesterol. Various

group of synthetic steroids plays a crucial role in medicinal value through their use as contraceptive drugs (Lopez *et al.*, 2014), cardiovascular agents (Rattanasopa *et al*., 2015), antibiotics, anesthetics, and anti-inflammatory (Aav *et al*., 2005).

2.3. Antimicrobial activity evaluation of plant extracts

Recently, researchers have been interested on the investigation of pure secondary metabolites compounds, essential oil, and newly synthesized molecules from plant extract as significant antimicrobial agents. Antimicrobial susceptibility evaluation methods or test is important for drug discovery, epidemiology and therapeutic outcome prediction. A variety of laboratory methods can able to screen or evaluate *in-vitro* antimicrobial activity. Among them, the most known and basic is discussed as below:

2.3.1. Agar disc diffusion method

Agar disc diffusion testing was developed in 1940. It is used with Muller Hinton agar for the determination of antimicrobial activities of the crude plant extracts. This method is not appropriate in order to obtain or determine the minimum inhibitory concentration. Because it is unable to quantify the amount of the antimicrobial agent diffused into the agar medium. This technique offers numerous advantages over other assay, like its simplicity, low cost, potential or capability to test a number of microbes and antimicrobial agents as well as ease to interpret the desired result (Balouiri *et al*., 2015).

2.4. Antioxidants

An antioxidant is the constituent of plant materials that act as radical scavengers and contributes to converting radicals to less reactive species. Various types of antioxidants are found in dietary sources like vegetables, fruit, and tea (Mandal *et al.,* 2009).This is playing a crucial role both in the food system and the human body to mitigate oxidative processes and the harmful effect of ROS (Çakmakçi *et al*., 2015). Antioxidant compounds can improve shelf-life by retarding the process of lipid peroxidation and scavenging or inhibiting free radicals which are the major reasons for the deterioration of food and pharmaceutical products at the time of processing and storage. In the food system, the involvement of nutritional antioxidant molecules is also able to prevent the formation of secondary lipid peroxidation products by maintaining the color, aroma, and texture of the food product during storage (Çakmakçi *et al*., 2015). Furthermore,

antioxidants also can protect the human body from free radicals and ROS effects. They delay the progression of chronic diseases, cancer, and age-related diseases such as coronary heart disease and prevent the radical chain reactions of oxidation when added to food (Gulcin, 2020). Phenolics and flavonoids are the major bioactive compounds of these natural sources which are responsible for their health benefits.

2.4.1 Antioxidant capacity evaluation

Antioxidant capacity is the overall ability of an organism or food to catch free radicals and prevent their harmful effects. In order to determine the antioxidant capabilities of various plant parts and their derivatives, a variety of tests have been routinely used. These are DPPH (2,2 diphenyl-1-picrylhydrazy), FRAP (ferric reducing antioxidant power), ORAS (oxygen radical absorption capacity), and ABTS. Among them, DPPH is discussed below.

2.4.1.1. DPPH free radical scavenging antioxidant activity

2,2-diphenyl-1-picrylhydrazyl is a stable free radical with purple color, which shows hydrogen acceptor ability towards antioxidants. Hence, it is commonly used in DPPH assay for measuring the antioxidant activity of different natural samples such as wine, fruits, herbal tea, etc. It is involving the use of free radical DPPH, which is widely used to test the ability of compounds to act as free radical scavengers/ hydrogen donors and to evaluate the antioxidant activity (Chekuri *et al*., 2018). The reduced DPPH-H is formed when a free radical scavenger pairs the odd DPPH radical electron with hydrogen. Up on reacting with antioxidants, produces corresponding hydrazine DPPH-H and it becomes pale yellow color. A strong absorption spectrum is caused by the DPPH free radical's odd electron at 517 nm.

2.5. Nanotechnology

Nanotechnology is a modern field of science that can be defined as the manipulation of materials at the atomic level. Nanotechnology involves the manufacturing, manipulation and imaging of nanostructures with sizes between 1 to 100nm scale (Kumar *et al*., 2017). The nanotechnology term was first used by Feynman in 1959 which is considered as the mark of beginning the of modern nanotechnology. The study of the modern concept of nanotechnology has been started widely in the nineteen centuries. This modern science has created new opportunities in various

areas/fields such as animal husbandry, agriculture, electronics, food packing, leather industries, medicine, pharmaceutical and health care (Anjum *et al*., 2021; Malik & Muhammad, 2023).

2.5.1. Green Synthesis Silver nanoparticles

Silver nanoparticles can be synthesized by physical, chemical and biological methods. In the biological method of nanoparticle synthesizing the use of microorganisms and plant extract/biomass as reducing agents/capping can substitute other procedures with various advantages due to their specific characteristics. Specifically, the employment of the plant extract in green synthesis is the most important than another biological processes because of their capability to reduce metal ions and produce stable nanoparticles easily and effectively in a short period of time. Whereas physical and chemical methods have faced several limitations like, expensive, time-consuming processes, use of hazardous reagents, their output has been toxic to the environment and not applicable in medical fields (Patra & Baek, 2014).

Metal nanoparticles synthesized by using biological sources/materials like fungi, bacteria, yeast and plants have been known by low-cost, energy-efficient, non-toxic, free of hazardous chemicals, care for medical and health care purposes (Ahmed *et al*., 2016; Sanyasi *et al*., 2016). Plant-based synthesis of nanoparticles is more important than other biological methods and can able to scale up for large-scale synthesis of nanoparticles. Various types of phytochemicals of plants like terpenoids, alkaloids, phenolic acid, polyphenols and proteins play a crucial role through served as capping and stabilizing agents in the reduction of metal ions into the nanoparticle and maintaining the stability of nano-size particles (Lee *et al*., 2014).

2.5.1.1 Application of green synthesized silver nanoparticles

The silver nanoparticle has been more widely studied than other metal nanoparticles/profound interest of researchers, due to its unique characteristics like size and shape depend on magnetic, optical and electrical properties which can help to be incorporated into biosensor materials, antimicrobial applications, electronics component, composite fibers, cryogenic superconducting materials and cosmetic products. Silver nanoparticles and other nanoparticles profound great interest due to their small size and large surface-to-volume ratio, which leads to both chemical

and physical variety in their properties compared to the bulk of the same chemical constituents (Xu *et al.*, 2020). Therefore, synthesizing and designing nanoparticles with their novel application can be resulted by controlling the shape and size at the nanometer scale.

These particles have numerous applications in different fields including drug delivery, medical imaging, nano-composites, filters, hyperthermia of tumors, environmental treatment, fuel batteries for storage of energy, in cosmetics and clothes (Jacob *et al.*, 2019; Kumar *et al*., 2019). Specifically, AgNps have drawn/influenced/ received the attention of researchers because of their huge role in several areas such as integrated circuits, sensors, applications of silver nanoparticles in biolabeling, filters, silver nanowires (low-cost paper batteries and antimicrobial agents (Krishnanand & Sekhar, 2018). Silver nanoparticles have been employed extensively as antimicrobial agents in medical areas, food storage, textile coating and a number of environmental applications.

The antimicrobial properties of AgNps made the use of these nanoparticles in different fields of medicine, several industries (textile, diaper), animal husbandry, food packaging, accessories, cosmetics, health and military (Cho *et al*., 2005; Rahman *et al*., 2016). This vast application of silver nanoparticles has led the recent development of green synthesized AgNps with many therapeutic effects. Moreover, the number of recent literature demonstrated that biosynthesized silver nanoparticles have exhibited that anticancer, anti-inflammatory, wound healing, bioimaging, antidiabetic, biosensing, anticoagulating and neurodegenerative properties (Kotcherlakota *et al*., 2019).

Silver nanoparticles have a high potential of antimicrobial activity. Recently, numerous studies reported the antimicrobial activity of green synthesized silver nanoparticles. Specifically, different parts of medicinal plants have been studied for the antibacterial effects of silver nanoparticles synthesized from them. Because, when silver nanoparticles synthesized by using these plants, have exhibited potent antimicrobial activities. Green synthesized silver nanoparticles from leaves extract of Pu-erh tea has been showed potential antibacterial effects on selected foodborne pathogens including *E. coli, K. pneumoniae, Salmonella Typhimurium and Salmonella Enteritidis* at low concentration (Loo *et al*., 2018).

AgNps prepared from extracts of whole parts of *Carduus crispus* have been screened for their antibacterial activity on both gram-positive (*M. luteus*) and gram-negative (*E.coli*) exhibited 7 ± 0.4 mm to 7.7 ± 0.5 mm and 5.5 ± 0.2mm to 6.5 ± 0.3mm respectively (Urnukhsaikhan et al., 2021). Other studies found that the antibacterial activity of AgNps synthesized from the root extract of *Salyadora persica* L on selected bacterial strains (Shaik *et al*., 2020). Furthermore, Green synthesized silver nanoparticles also have been studied for their antifungal activity. AgNps prepared from leaves and flowers extract of *Teucrium polium* L (Felty germander) have showed against the growth of *F. oxysporum* through inhibiting colony formation (Ghojavand *et al*., 2020).

2.6. Description of the selected study

2.6.1 Genus Verbascum

Scrophulariaceae family is a large family containing around 300 genera and 5400-5500 species and distributed throughout the world. *Verbascum* genus is the dominant genus which contains about 360 species. It is represented in Ethiopia by nine species such as *Verbascum arbusculum, Verbascum benthamianum, Verbascum chiovendae, Verbascum pubescens, Verbascum scabridum, Verbascum sedgwickianum, Verbascum stelurum, Verbascum valerianifolium and Verbascum sinaiticum* (Fullas, 2010; Worku , 2016).. They have various medicinal values. Their leaves and flowers have expectorant, mucolytic and demulcent properties and are used to treat various diseases or respiratory disorders like bronchitis, dry coughs, tuberculosis, and asthma in Turkey's traditional medicine (Tatli & Akdemir, 2006). In addition, also contributed to folk medicine as a diuretic, antirheumatic, for wound healing, ophthalmic, against cold and chest diseases (Ahmed *et al*., 2015). Verbascum species contain various bioactive compounds including flavonoids, phenylethanoids, neolignan glycosides, saponins, and iridoid and monoterpene glycosides (Tatli & Akdemir, 2006).

Numerous classes of natural plant-derived compounds such as flavonoids, phenolic acids, sterols, triterpenes, saponins, polysaccharides, alkaloids, and iridoid glycosides were detected in Verbascum species (Mahmoud et al., 2007). Verbascum species have been shown various pharmacological activities such as antiviral activity, antimicrobial, antimalarial activities,

antioxidant activity, anti-inflammatory, antinociceptive activities, antitumor, anticancer and cytotoxic activities, immunomodulatory activity due to the presence of different types of biologically active compound in the species of this genus (Tatli & Akdemir, 2006).

The extracts obtained from various species of Verbascum such as *Verbascum olympicum* Boiss, *Verbascum prusianu* Boiss, and *Verbascum bombyciferum* Bois have shown against selected species members of gram-positive bacteria and yeasts, Whereas, They have no antibacterial activity on tested gram-negative bacteria (Dülger *et al.*, 2002). Numerous studies suggested that flavone groups like luteolin and flavanols have neuroprotective activity. Since these species of plants are used for the treatment of neurodegeneration diseases (Tatli *et al*., 2015).

2.6.2. *Verbascum sinaiticum Benth* description and taxonomic classification

V. sinaiticum is a biennial herb plant with 60 -150cm or reaching up to 2m in height. It is ordinarily found in Turkey, Eastern Africa, and Arabian Peninsula. Particularly grows in a wide range of geographic areas of Ethiopia(Abebe, 2016). The whole plant is densely shaggy and grayish and its leaves are simple and broadly ovate-oblong (Umer *et al*., 2010). It is known in different parts of Ethiopia by the local name; Gurra Harree' in Afan Oromo, Ayajoro or Qetetina or Yefaras Zeng in Amharic.

Figure 1: **Photograph of *V. sinaiticum* plant** (from sampling area)

Table 1: Taxonomical classification of *Verbascum sinaiticum*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Scrophulariaceae
Genus	*Verbascum* L.
Species	*Verbascum sinaiticum*

2.6.3. Traditional medicinal values of *V. sinaiticum*

The leaves, flowers, and roots of *Verbascum Sinaiticum* have various medicinal values. Traditionally the leaves of this plant is used to cure or treat various diseases such as postpartum diarrhea, haemorrhage, measles, anthrax, wound, superficial fungal infection, and elephantiasis(Tadeg *et al*., 2005; Wondimu *et al.*, 2007; Lulseged *et al*., 2022). Its root also contributed to the treatment of anthrax (Tadesse *et a*l., 2018) allergic dermatitis, the evil eye(Eyenewog)(Yohannis *et al*., 2018), syphilis, gonorrhea, mental illness, amnesia, tape warm, relapsing fever, rheumatic pain, elephantiasis and abdominal dropsy (Yeabyo *et al*., 2018). Roots and flowers have been involved in the treatment of fungal infections.

Furthermore, *Verbascum sinaiticum* is traditionally used for the treatment of stomachache (Chekole, 2017) (Teklehaymanot *et al*, 2007), hepatitis(Yineger *et al*., 2007), viral infections like 'Almaz belachira', cancer (neqersa) via applied topical by mixing powder of plant with hyena faces and latex (Teklehaymanot, 2009) and powder of the leaves *V. sinaiticum* mixed with + water is given orally or the filtrate is instilled into left ear or nose for the treatment of animal trypanosomiasis (Weldegerima *et al.,* 2008). This plant is not only used to treat human diseases but also for the treatment of animal ailments such equines bloat(Yehaya hodmenfat, cattle cough or pneumonia, etc. (Tadesse *et al.*, 2018; Weldegerima *et al*., 2008). Moreover, it is used for the treatment of liver diseases, tumors, stomach troubles, diabetes, scabies, amoebiasis, diarrhea, epilepsy, and cough in Ethiopian traditional medicine (Ahmed *et al*., 2015).

2.6.4. Biological activities of *V. sinaiticum* Benth

Studies of antimicrobial evaluation of medicinal plants are important revealing locally available plant species and discovering crude drugs. Various studies investigated the antimicrobial activities of *Verbascum sinaiticum* (Scrophulariaceae) both on fungal and bacterial strains which are recognized to be skin pathogens (Tadeg *et al*., 2005). Acetone and ethanol root extract of *Verbascum sinaiticum* has been exhibited against both tested gram-negative and positive bacteria (Yeabyo *et al.*, 2018). Due to the presence of various secondary metabolite compounds includes; terpenoids, alkaloids, flavonoids, saponins, tannins, phenolic compounds, cardiac glycosides, and steroids remaining phytoconstituents coumarins.

The Leave extract of this plant also has been shown in vitro broad-spectrum antimicrobial activity. Methanol extract of *Verbascum sinaiticum* leaves against species of both Gram-positive and negative bacteria which include *S. aureus*, and *P. aerugonisa* respectively (Tadeg *et al*., 2005). Similarly, Aqueous and methanol leaf extract of *Verbascum sinaiticum* have also exhibited *in vitro* antitrypanosomal activity due to the presence of numerous phytochemical compounds in a plant (Mergia *et al.,* 2014). Other studies from Turkey also showed that ethanol leaf extract of *Verbascum sinaiticum* revealed strong antimicrobial activities against the pathogens isolated from urinary tract infections (Sener & Dulger, 2009).

In this study, *Enterococcus faecalis* and *Candida albicans* have been shown broad spectrum zone of inhibition than standard antibiotics including Ampicillin and streptomycin as antibacterial while nystatin is the antifungal positive control. According to Afifi*etal*. (1993) reported the presence of flavonolignans, hydrocarpin, and the novel *sinaiticum*, as well as two flavones, chrysoeriol, and luteolin from a phytochemical screening of leaves *V. sinaiticum* which have been shown dose-dependent cytotoxicity against murine Leukemia cell line. 80% Methanol leaf extract of *Verbascum sinaiticum* has exhibited high antioxidant activities or radical scavenging properties in DPPH assay in IC_{50} at 1.70mg/ml (Lulseged *et al*., 2022).

3. Materials and Methods

3.1. Description of the study area

The leaves of *Verbascum sinaiticum* were collected from the Wachale district North Shewa Oromia regional state of Ethiopia. It is located at the North of Addis Ababa, far away 78km and 34km from Fiche which is the capital city of the north Shewa zone, Oromia regional state. The highest and lowest elevation of the study are 2880m and 1200m respectively. The geographical location of the study area lies between the average elevation of 2,412 m above sea level at an altitude of 9^0 25’ 2.13” to 9^0 48’44” N and a Longitude of 38^0 38’ 49.02” to 39^0 08’41” E. The mean annual rainfall of the district is ranged from 1000mm to 1800mm and the average temperature is 13.5^0C to 20^0C. The soil type of the district falls into strongly acidic, moderately acidic and neutral, as well as three major soil types such as black, brown, and red soil (Bedada *et al*., 2020). It has 48,880 hectares of the total area (Bedada *et al*., 2020).

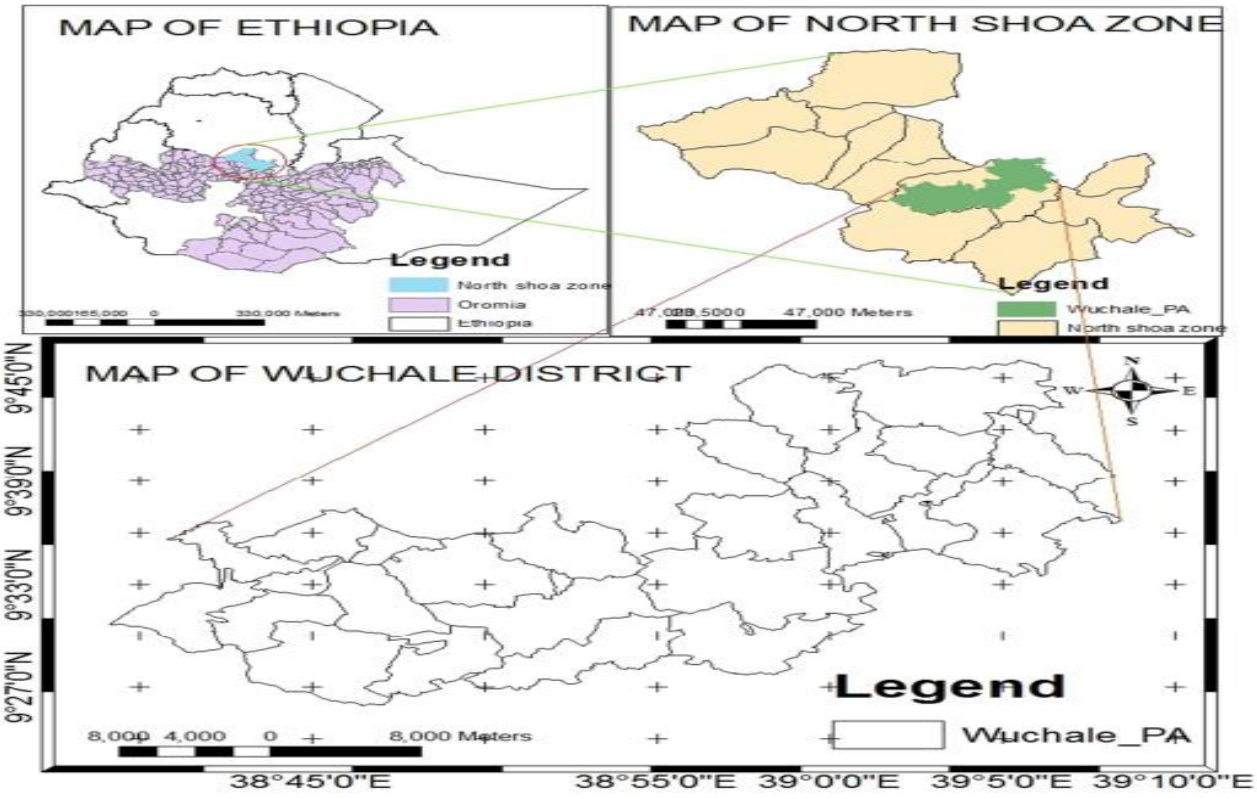

Figure 2: Map of the study area (Bedada *et al*., 2020)

3.2. Collection and authentication of plant material

A plant sample for plant identification was collected in January 2023 from Wachale Weroda North Shewa Oromia regional state of Ethiopia and the plant material was taxonomically at national Herbarium, Addis Ababa University. The specimen of plant material was preserved with KH001 voucher code/number was deposited in the National Herbarium of Addis Ababa University.

3.3. Chemicals and reagents

The chemicals and reagents used for this research were distilled water, methanol, chloroform, acetone, n-hexane, Ferric chloride, Wagner's reagent (Iodine in potassium), sulfuric acid (H_2SO_4), ammonium solution, DMSO, MHA, SDA, acetic anhydride, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Sodium chloride, $AgNO_3$ and other chemicals.

3.4. Sample preparation

3.4.1. Extraction process and Crude extract preparation

The leaves of *Verbascum sinaiticum* were collected from the study area and washed thoroughly with running tap water to remove dirt, dust or unwanted material. The cleaned plant sample was cut into small parts to increase volume surface contact with solvents, facilitate drying and air dried at room temperature under shade for a week until well dried for grinding. The dried leaves were grinded to powder using an electrical grinder and the powder was packed in a plastic bag and stored for extractions. The powdered plant leaf sample was weighted by using an electrical balance. The leaf powder (100g) of *V.sinaiticum* was macerated with 1L water, chloroform, acetone, n-hexane and methanol solvents separately and stored for 72hrs with occasional shaking at room temperature (Abubakar & Haque, 2020). Then, the extracts were filtered by Whatman no. 1 filter paper from residue and the extracts were concentrated using a rotary vacuum evaporator to obtain the dried powdered extracts. Extracts were stored in closed and dark places until used for further analysis or future experiments. The percentage yields of the crude extracts were calculated by the following formula:

Percentage yield of the extract (%) = Crude extract/the dried powdered leaves x100%

Figure 3: Diagrammatical representation of sample preparation

3.4.2. Preparation of plant extract for AgNps synthesis

The leaves of *V. sinaiticum* were collected from the above-mentioned sampling area. Then washed three times, first with taping water to remove dirty materials and next washed by using double distilled water two times to make plant materials completely clean. Next plant sample was cut into small sizes and air-dried for two weeks. After the drying period, the leaves of *V. sinaiticum* became powdered by using electrical grinding. The powder plant leaf weighted and 40g was macerated with 400 ml of doubled distilled water in the 600 ml beaker and put in a shaker incubator at 25^0 C and 120rpm for three days or 72 hours. Then, the plant extract was filtered by using Whatman No. 1 filter paper and the filtrate was stored at 4^0C in the refrigerator until used for silver nanoparticles synthesis.

3.4.3. Preparation of Silver Nitrate solution

Silver nitrate solution was prepared from commercially purchased $AgNO_3$. 1mM concentration of silver nitrate was prepared by dissolving 0.0339g of silver nitrate in the 200ml of doubled distilled water.

3.4.4. Synthesis of Silver nanoparticle

$AgNO_3$ and plant extract were used as precursors for silver nanoparticle synthesis. In order to synthesize AgNps, 50ml 1mM of AgNO3 was dissolved with 200ml of aqueous plant extract as described previously by Apu *et al*., (2022). Each mixture of plant extract and $AgNO_3$ solutions were heated at 60^0C on a stirrer hot plate with a magnetic bar for 1hr 15min/20min. Color change observed in several periods of time was recorded. The protocols used in synthesize of silver nanoparticles was done as previous studies (Jemal *et al*., 2017) with slight modifications. The mixture solution was repeatedly centrifuged at 13,000rpm for 10min by washing with alcohol and distilled water. After, the end of centrifugation, the sample was disposed from the centrifuge tube by using ethanol into crew sepal and placed in the oven overnight in order to dry AgNps in a dark place. Finally, the dried AgNps were powdered by using a sterilized mortar and pestle and transferred to a sample holder, stored for further characterization, antimicrobial and antioxidant tests in a dark place

3.5. Qualitative analysis of phytochemicals

Different types of phytochemical tests were performed for qualitative analysis of secondary metabolites or phytochemicals in the leaf extract of *Verbascum sinaiticum.*

3.5.1. Detection of flavonoids

Ammonium tests: 2ml of extracts were treated with 2ml of dilute ammonia solution and a few drops H_2SO_4 were added. The presence of yellow color indicates the presence of flavonoids (Kumar et al., 2013).

3.5.2. Detection of phenolics

Ferric chloride test: 2ml of extracts were treated with 3-4 drops of ferric chloride solution. The formation of bluish-black color indicates the presence of phenolics (Pandey & Tripathi, 2014).

3.5.3. Detection of alkaloids

Wagner's test: Few drops (2ml) of Wagner's reagent were added to 2ml of plant extract along test tubes and a formation of reddish-brown precipitate confirm a positive test of alkaloids (V. Singh & Kumar, 2017).

3.5.4. Detection of saponins

Froth test: the extract was diluted with 20ml of the distilled water, and was shake in graduated cylinder for 15 minutes. The formation of 1cm foam is indicate the presence of the saponin (Pandey & Tripathi, 2014).

3.5.5. Detection of tannins

Ferric chloride test: 1 ml of the extract was mixed with 2 ml of distilled water. Then, 2ml of 10% $FeCl_3.H_2O$ were added to the mentioned mixture. The formation of greenish-brown or dark-green color confirms the presence of tannins (Shafodino *et al*., 2022).

3.5.6. Detection of steroids

Salkowski's test: 1ml of each extract is treated with 2ml of chloroform and treated with few drops of concentrated H_2SO_4. The appearance of red colour on lower layer of chloroform indicates the presence of the steroids (Ram & Sinha, 2015).

3.5.7. Detection of terpenoids

Salkowski's test: 2ml of chloroform was to 1ml of each extract and 3ml of concentrated H_2SO_4 were carefully added and mixed well. The formation of red-brown color confirms the presence of terpenoids(Yadav & Agarwala, 2011;Shafodino *et al*., 2022).

Liemermann-Burchard test: 2ml of chloroform and acetic anhydride were treated with 1ml of extract, then few drops of concentrated H_2SO_4 was added. Finally, the formation dark-green colour confirms the positive test of terpenoids (Tyagi, 2017).

3.5.8. Detection of glycosides

Small amounts of extracts were dissolved/treated with 1ml of distilled water and aqueous NaOH solution was added. Finally, the formation of yellow colour was confirms the presence of glycosides (Kumar & Jat, 2018; Ray *et al*., 2013).

3.6. Antimicrobial assay of the leaf extract

3.6.1 Test microorganisms

Microorganisms selected for this study were two gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), two gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*) and one fungus (*Candida albican*) which were obtained from Oromia regional laboratory, Adama.

3.6.2. Inoculum preparation and standardization

McFarland standard is a chemical solution of Barium chloride and sulphuric acid, which result in the formation fine precipitate, barium sulfate. McFarland was shaken well and aliquoted in test tubes identical to those used to prepare inoculum suspension. Before each use shake well is important for barium sulphate to be evenly distributed in the solution. 0.5 McFarland standard is prescribed for antimicrobial susceptibility tests. This standard was prepared by mixing 0.05ml $BaCl_2$ (1.17% w/v $BaCl_2$. $2H_2O$) 9.95 ml %1 w/v H_2SO_4 in distilled water, to standardize the approximate number microbial in liquid suspension by comparing the turbidity of test with standard (Hudzicki, 2016; Mukhtar *et al*., 2017).

3.6.2.1 Inoculum preparation for bacteria

Each bacteria species was sub cultured on Muller Hinton agar and incubated for 24 h at 37^0C. The inoculum suspension was prepared by picking several colonies (three to five colonies) of a fresh culture of Muller Hinton agar (MHA) with a sterile inoculating loop and transferred into a test tube containing 5ml of MHB and incubate overnight. Then fresh cultures were diluted by adding sterile saline solution and vortexed thoroughly. This was repeated until matched with the turbidity 0.5 McFarland standard corresponding to 1.5×10^8 CFU/ml for bacterial inoculum suspension (Andrews, 2001; Wiegand *et al*., 2008).

3.6.2.2. Inoculum preparation for fungi

Fungal was sub-cultured on Saboured Dextrose agar and incubated for 48 h at 27^0C. The inoculum suspension was prepared by picking several colonies (three to five colonies) of the fresh culture of Saboured Dextrose agar (SBA) with a sterile inoculating loop and transferring into a test tube containing 5ml of SDB and incubated overnight. The fresh culture was diluted with saline solution and vortexed thoroughly. Then fungal isolate was adjusted to turbidity 0.5

McFarland standard corresponding to $1x10^6$ cell/spores for fungal species (Andrews, 2001; Wiegand *et al*., 2008).

3.6.3. Antibacterial and antifungal evaluation of the extract

Disk diffusion assay is the standard method used to determine the antimicrobial activity of antimicrobial agents as standard procedures developed previously (Hudzicki, 2016). This method was used to determine the antimicrobial activity of methanol, distilled water, acetone and chloroform crude extract of *V. sinaiticum* leave on five selected pathogenic microbes. As mentioned above, the microbial inoculum was sub cultured into the MHB for bacterial strains and Sabroud Dextrose broth for fungal strains and incubated overnight to get fresh culture. Then, these inoculums were diluted in order to adjust with 0.5Mcfarland standard as mentioned above. Microbial inoculum suspensions were taken by sterile cotton swab and streaked/spread on sterile muller Hinton agar and SDA plate for bacterial strains and *C. albican* respectively. 200mg/ml of stock solution of each crude extract was prepared by dissolving 0.4g of methanol, distilled water, acetone and chloroform in 2 ml of 10% DMSO. Two-fold serial dilution (200mg/ml, 100mg/ml, 50mg/ml, 25 mg/ml) of each crude extract was formed for disc diffusion assay. Additionally, for determination of antimicrobial activity green synthesized silver nanoparticles, 20mg/ml stock solution of AgNps were prepared and two-fold serial dilutions (10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml) were formed from this stock solution. Approximately, 6mm diameter of Whatman No.1 filter papers were prepared by using a puncher and sterilized by an autoclave for this test. These sterile 6mm discs were soaked in each two-fold serial concentration of crude extract and green synthesized AgNps of *V. sinaiticum* leaf for 20min minutes. Sterile paper discs soaked in 30µg/ml Ciprofloxacin and ketoconazole standard antibiotic solution were used as the positive controls. Whereas, discs impregnated with DMSO were used as the negative control. Next, all these discs were placed by using sterile forceps on sterile agar plates (MHA and SDA) which spread with a fresh culture of microbial strains (bacterial strains and *C. albican*) in the laminar airflow cabinet on their labeled location and the plates were left for 30min in the hood for the diffusion of the antimicrobial agent from the disc into a plate. Then, plates were inverted and incubated for 24 h at 37^0C and for 48 hrs. at 27^0C for bacterial and fungi growth respectively. After the end of incubation time, the inhibition zone of the microbes by microbial

agents was measured by using a rural millimeter scale. The tests were done in triplicate and the final result was presented as an arithmetic average mean (mean ± SD) (Buli *et al*., 2015).

3.6.4. Determination of minimum inhibition concentration (MIC)

The minimum inhibition concentration was determined by the broth dilution method (Balouiri *et al*., 2015). In order to determine MIC by broth dilution method, early adjusted microbial suspensions were diluted to 5×10^5 CFU/ ml and 2.5×10^4 CFU/ ml for bacterial and fungal (Wiegand *et al*., 2008). 200mg/ml of stock solution of each crude extract of leaves of *V. sinaiticum* was prepared. Two-fold serial dilution of each extract was prepared as 100, 50, 25, 12.5 and 6.25 mg/ml in 10ml beakers. Similarly, a 20mg/ml stock solution of green synthesized AgNps was also prepared and two-fold serial dilution was formed (10, 5, 2.5, 1.25 and 0.625 mg/ml) for the same purpose. 100 µl of each crude extract and solution of AgNps were added/ transferred by using a micropipette into the test tubes containing 5ml of MHB and SDB which were marked with their respective concentrations of crude extract and AgNps for all selected pathogenic microbial inoculum. Then, 10 µl microbial (*E. coli, S. aureus, S. pyogenes, P. aerogenes and C. albican*) inoculum adjusted to 0.5Mcfarland were added to each marked test tube according to the previously adopted method with slight modifications (Andrews, 2001; Mukhtar *et al*., 2017). Two test tubes were used as negative/broth inoculated with inoculum/ and positive controls/broth only/ for each tested microbe. Next; Finally, all test tubes were incubated according to standard microbial incubation period and temperature to examine their growth (CLSI, 2015). After the end of the incubation period, the lowest concentration which inhibited the microbial growth was recorded as MIC value by visual observation/optically for each crude extract and AgNps. Additionally, in the case of MIC value determination for green synthesized AgNps the turbidity of tested microbial strains was measured at 600nm by using the UV spectrophotometer (Dahiya & Purkayastha, 2012). The minimum concentrations with low microbial turbidity were taken as MIC values.

3.6.5. Determination of minimum bactericidal and fungicidal concentration

Minimum bactericidal and fungicidal concentrations were evaluated after determination of MIC values. These were done by subculturing the aliquot of samples from test tubes that didn't show visible growth of bacterial and fungal species from determination of the MIC, on the surface of

MHA and SDA plates. Each subculture was incubated for 24 h at 37^0C and 48h at 27^0C for bacteria and fungi respectively. Finally, after the end of the incubation period, the lowest concentration of each plant extract and green synthesized silver nanoparticles which prevent the growth of bacterial cells and fungal cell were considered as MBC and MFC values (Akinduti *et al*., 2019).

3.7. DPPH radical scavenging activity

The antioxidant activity of plant extracts was evaluated based on the free radical scavenging activity of 2, 2- diphenyl-1-picrylhydrozyl (DPPH). The free radical scavenging activity of extracts was determined according to the method developed by Brand- William (Brand-Williams *et al*., 1995) with slight modifications (Pranoothi *et al*., 2014). A 0.004% methanol solution of DPPH was prepared. 1ml DPPH solution was added to 3ml series concentration of (50, 100,150, 200,250) µg/ml of leaf extract of methanol, water, acetone, chloroform and green synthesized silver nanoparticles in separate test tubes. Similarly, DPPH solution was added to series concentrations of ascorbic acid (50, 100, 150, 200) µg/ml. Ascorbic acid was used as a reference and DPPH in a methanol solution is used as a control. The solutions were mixed well and incubated at room temperature for 30min in the dark (wrapped with aluminum foil). Then, absorbance was measured at 517nm by using a spectrophotometer. The percentage of scavenging activity of the extracts or percentage of inhibitions was calculated by the following formula:

% inhibition= (Ao-A1)/Ao × 100

When, Ao- Absorbance of the control reaction; A1- absorbance of the sample or all the extracts and standard

Dose-response curves were plotted between the percentage of scavenging activity(y-axis) and various concentrations of plant antioxidants (x-axis) by using Microsoft word excel 2016. IC_{50} was the inhibition concentration of the extract that scavenge or delay the formation of the DPPH radicals by 50%. IC_{50} value was calculated through linear regression plots of concentration versus the percentage of DPPH radical scavenging analysis.

3.8. Characterization of green synthesized AgNps

Green synthesized AgNps was characterized by SEM, XRD and UV spectrophotometer.

3.8.1. Visual observation of color change

Green synthesized silver nanoparticles were preliminarily characterized by visual observation of gradual color change of reaction mixtures of aqueous plant and silver nitrate solution.

3.8.2. Scanning electron microscopy

Scanning electron microscopy was used for the analysis/characterization morphology of green synthesized silver nanoparticles. shape, size and crystalline nature of AgNps were determined by Scanning electron microscope (Karthiga, 2018).

3.8.3. X-ray diffraction

XRD analysis was employed for the characterization of the crystalline nature of synthesized silver nanoparticles. The crystalline nature of green synthesized AgNps was characterized by an X-Ray diffractometer (SHIMADZU XRD-7000 X-Ray diffractometer, Japan) with 40KV, 30mA, and 1.5406A° Cu K*a* radiation source in 2θ was used. The XRD data were recorded in terms of intensity of diffraction versus 2θ. The XRD pattern of the sample was plotted by using Origin pro software. The result of the XRD pattern of AgNps was compared with the standard compiled by Joint Committee on Powder Diffraction and Standards (JCPDS) pattern to exhibit its consistency.

3.9. Data analysis

For disc diffusion assay, the experiment(test) was replicated three times and the results were presented as mean ± SD. Statistical Package of Social Science (SPSS) 22.0 version was used to analyze the results of Diameter of zone of inhibition of each extract and green synthesized silver nano particles of *V. sinaiticum* leaf. The experimental data were entered into IBM SPSS statistics data variable view. The descriptive statistics were performed for calculation mean group of zone inhibition as mean ± SD. One-way ANOVA was employed to determine the significance among the group means. One-way ANOVA followed by Tukey's Post Hoc Tests were carried out to compare the result obtained from different mean group and determine the statistical significance between each group mean. P values less than 0.05 were considered as statistically significant.

4. Results

4.1. Crude extract yield

In this study the leaf of *V. sinaiticum* was macerated with polar (methanol and distilled water) medium(acetone) and non-polar solvents (chloroform and n-hexane) were used in the extraction process of phytochemicals. The reason for using these five solvents was to screen/investigate various types of bioactive compounds with different levels of polarity. This means the plant-derived compound having polar properties was extracted via polar solvents, while those with nonpolar properties were extracted by using non-polar solvents. The final percentage yield of the methanol, distilled water, acetone, chloroform and n-hexane crude extracts of leaves of *V. sinaiticum* were determined as shown in below **Table 2**. The Polar solvents like methanol, water and medium polar/ acetone were extracted high yield of crude extracts from the leaf part of *Verbascum sinaiticum* than non-polar solvents (n-hexane and chloroform). Therefore, the yield of the crude extract relied on the degree of polarity of the solvents used for extraction.

The percentage yield of the extract (%) = Weight of crude extract/Weight of the dried powdered leaves x100%

Table 2: Yield of crude extracts of *V. sinaiticum* leaf

No	Solvents	Weight of powder(g)	Weight of crude(g)	Percentage of yield (%)	Colour of crude extract
1	Methanol	100	8.3	8.3	Dark green
2	Distilled water	100	6.85	6.85	Black brown
3	Acetone	100	5.6	5.6	Yellowish black
4	Chloroform	100	3.27	3.27	Black green
5	n-hexane	100	0.4	0.4	Yellow

4.2. Qualitative phytochemical tests

Methanol, distilled water, acetone, chloroform and n-hexane crude extract of *Verbascum sinaiticum* were screened for qualitative tests of bioactive compounds like phenols, alkaloids,

flavonoids, saponins, tannins, terpenoids, steroids and glycosides. Among these phytochemicals, phenol was detected in a small amount only in methanol crude extract of *V. sinaiticum.* While, flavonoids, terpenoids and glycosides were present in the methanol, acetone, chloroform and n-hexane crude extracts. Alkaloids were also highly detected in all crude extracts except in acetone. Similarly, steroids were detected in all crude extracts, absent in distilled water. Saponin was absent in n-hexane crude extract. Nevertheless, Phenols were only detected in methanol crude extract. Generally, methanol solvent extracted a wide range of phytochemicals from the leaf part of *Verbascum sinaiticum.*

Table 3: Qualitative phytochemicals test analysis

No	Phytochemicals types	Types of tests/reagents	Water extract	Methanol extract	Acetone extract	Chloroform extract	n-hexane extract
1	Alkaloids	Wagner's	+	++	–	++	++
2	Flavonoids	Ammonium	+	+	++	+	+
3	Phenols	Ferric chloride	–	+	–	–	–
4	Steroids	Salkowski's	–	+	+	++	+
5	Terpenoids	Liemermann burchad & Salkowski's	++	++	+	++	+
6	Saponins	Froth	++	+	±	+	–
7	Tannins	Braymer's	+	–	++	–	–
8	Glycosides	Baljet's	–	++	+	+	++

Key: ++ highly present; + present; ± slightly present /present in small amount; - absent

4.3. Green synthesized silver nanoparticles

One millimolar of AgNps was prepared by adding the desired amount of plant extract into the silver nitrate solution. After the $AgNO_3$ solution and plant extract were mixed, the color change was observed in the period of time. As the reaction time of the mixture increase or stirring the mixture on the hot plate, a gradual color change from greenish yellow/pale yellow into reddish brown was observed. The formation of this color confirmed the synthesis of AgNps as shown in **Figure 4**

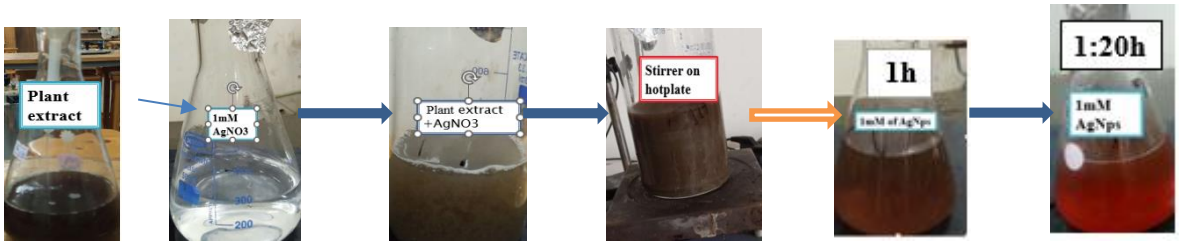

Figure 4: **Synthesis silver nano particles using aqueous leaf extract of *V. sinaiticum***

4.3.1 Visual characterization

Silver nanoparticles were synthesized from an aqueous leaf extract of *V. sinaiticum*. The mixture color changed gradually from greenish yellow to reddish brownish color that confirms the presence AgNps as given in **Figure 4**.

4.3.2. X- ray diffraction (XRD) analysis

XRD was used for the investigation of crystalline nature and structure of green synthesized silver nano particles. As a result, four peaks green synthesized silver nano particles were clearly observed from X-ray diffraction pattern (**Figure 5**). These four peaks obtained at 2θ angles with 38.18º, 44.45º, 64.426º and 77.47º values, for their corresponding diffraction planes 111, 200, 220 and 311 of the face cubic centered of silver nano particles respectively. The sharpness of peaks plane reflection/diffraction indicated the high intensity thus confirmed the positions of atomic in crystal structures. The result of XRD pattern was exactly matched with standard JCPDS file with card no 0040783. This confirmed synthesized AgNps were in crystalline nature with face cubic centered crystal structure.

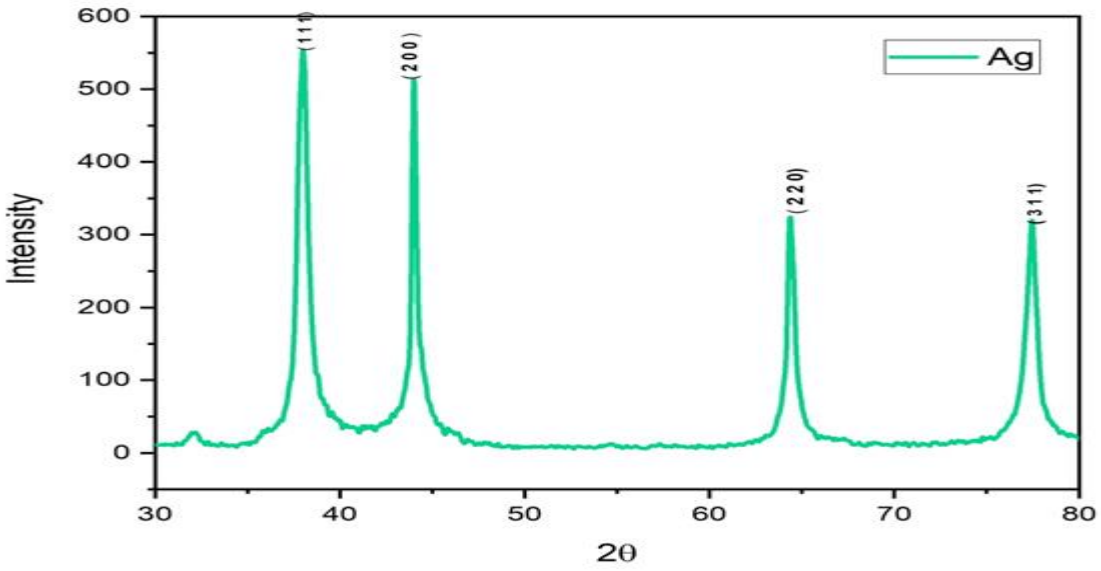

Figure 5: XRD of AgNps of *V. sinaiticum*

4.3.3. Scanning electron microscope analysis of AgNps

The size, shape and morphology of the green synthesized AgNps was characterized by using a Scanning electron microscope. SEM image of silver nanoparticles revealed, the shape green synthesized silver nanoparticles were dominantly spherical with high agglomeration of silver nano particles and presence various pores (**Figure 6**). The porosity indicated the presence active compound on the surface of silver nanoparticles. Average size of green synthesized AgNps was 47 to 80nm.

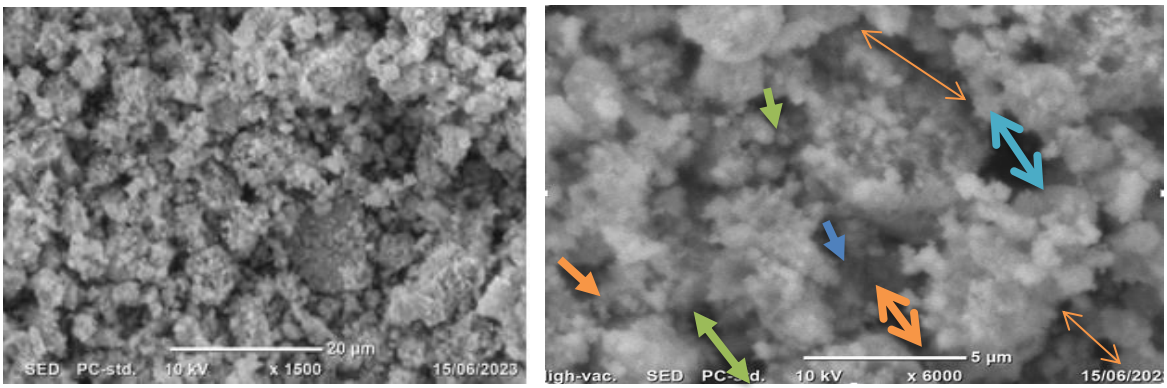

Figure 6: SEM of green synthesized silver nano particles (arrows indicate porosity)

4.4. Antimicrobial assay

4.4.1. Disc diffusion method

The invitro antibacterial and antifungal assay of plant crude extracts were determined by the paper disc diffusion method. Each crude extract and green synthesized AgNps of leaves of *V. sinaiticum* showed significantly potent antimicrobial activity on tested microbes as given below in **Table 4** and **Figure 7.** As summarized in **Table 4**, in the case of the antibacterial, methanol crude extract was produced/ exhibited maximum zone of inhibition range from 17±1.0mm on *S. aureus* to 9.67±0.57 against *E. coli.* Next, chloroform crude extract formed a significantly large zone of inhibition 16.0±1.0 and 15.67±0.57mm by retard the growth of gram-positive bacteria (*S. aureus* and *S. pyogenes*), Whereas, 13.0±1.0 and 14.0±1.0mm against *E. coli* and *P. aeruginosa* respectively. Acetone extract significantly produced zone of inhibition 15.3±0.57, 14.33±0.57, 12.33±0.57 and 13.0±0.57mm on against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* respectively. Distilled water extract produced maximum zone inhibition against *S. pyogenes* (14±1.52mm), while exhibited minimum zone of inhibition on *E. coli* (7.67±0.57mm). However, in the case of antifungal activity chloroform extract exhibited maximum zone of inhibition against *C. albican* with 13.0±1.0 to.8.0±1.0mm. Methanol extract showed zone inhibition ranged from 12.67±1.57 to 8.3±1.57mm. Acetone extract significantly exhibited zone of inhibition range of from 12.0±1.0 to 7.67±0.57mm. AgNps revealed maximum inhibition zone tested microbes as follows on *E. coli* (16.0±1.0mm)>P. *aerugonisa* (15.67±0.55mm)>*S. aureus* and *C. albican* (15.0±1.0mm)> *S. pyogenes* (14.67±0.5mm). In the case of antibacterial activity, green synthesized AgNps significantly exhibited maximum zone of inhibition on *E. coli* 16.0±1.0mm, whereas minimum zone of inhibition against *S. aureus* 10.0±1.0mm (zone of inhibition ranged from 16.0±1.0 to 10.0±1.0mm against *E. coli* and *S. aureus* respectively). In the case of antifungal activity, AgNps significantly showed maximum zone of inhibition ranged from 5.0±1.0 to 10.67±0.57mm against *C. albican.*

All results were given in the below Table 4.

Table 4: **Zone of inhibition of crude extracts and AgNps of *V. sinaiticum* leaf in M± SD**

Sample	Con (mg/ml)	Diameter of zone of inhibition in mm (M±SD)				
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albican*
Me	200	17.0±1.0	16.33±0.57	14±1.0	14.67±0.57	12.67±1.57
	100	14.33±0.57	14.67±0.57	12.0±1.0	12±1.0	11.33±0.57
	50	12.0±1.0	13.0±1.0	11.33±0.57	11±1.0	10±1.0
	25	10.0±1.0	11.0±1.0	9.67±0.57	9.67±1.1	8.33±1.57
Dw	200	14.0±1.0	14.33±1.52	12.0±1.0	12.33±0.5	11.0±1.0
	100	11.0±1.0	12.0±1.0	11.0±1.0	10.67±1.1	9.67±0.57
	50	10.0±1.0	11.0±1.0	9.67±0.57	10.00±1.0	8.67±0.57
	25	8.67±0.57	9.0±1.0	7.67±0.57	8.33±0.57	7.33±0.57
Ace	200	15.33±0.57	14.33±0.57	12.33±0.57	13.0±0.57	12.0±1.0
	100	13.0±1.0	12.33±0.57	11.67±1.5	11.33±0.57	11.0±1.0
	50	10.67±0.57	11.00±1.0	11.33±0.5	9.67±0.57	10.0±1.0
	25	9.33±0.57	9.0±1.0	8.0±1.0	8.0±1.0	7.67±0.57
Chlo	200	16.0±1.0	15.67±0.57	13.0±1.0	14.0 ±1.0	13.0±1.0
	100	13.67±0.57	13.0±1.0	11.67±0.57	12.33±0.57	11.0±1.0
	50	11.0±1.0	11.33±0.57	10.0±1.0	10.33±1.52	10.0±1.0
	25	9.67±0.57	9.0±1.0	8.33±0.57	8.67±0.577	8.0±1.0
AgNps	10	15.0±1.0	14.67±0.57	16.0±1.0	15.67±0.57	15.0±1.0
	5	12.67±0.57	13.0±1.0	13.67±0.57	13.33±0.57	13.0±1.0
	2.5	11.33±0.57	11.67±0.57	11.67±0.57	12.33±0.57	12.67±0.57
	1.25	10.0±1.0	10.67±0.57	11.0±1.0	10.67±0.57	10.67±0.57
Cipro	20µg/ml	21.0±1.0	21.33±1.15	20.0±1.0	20.33±1.52	-
Ketoc	20µg/ml	-	-	-	-	21.67±1.5
DMSO	1ml	0	0	0	0	0

Note: From the analysis of SPPSS One way **ANOVA 22.0 version**, the mean values were significant at $p<0.05$ value. Key; M ±SD stands for M- mean, S- standard, D-deviation.

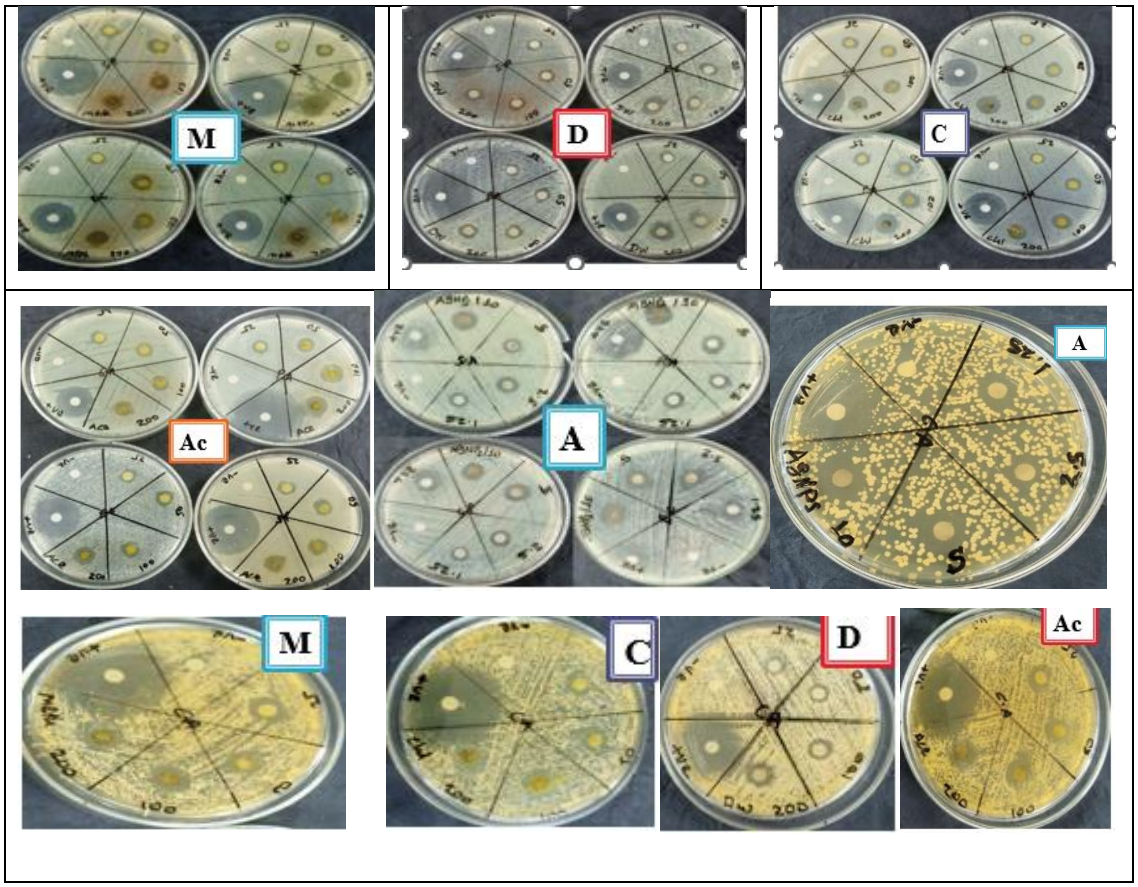

Figure 7: Photo of Disc diffusion of antimicrobial activity of extracts and AgNps

(**Key**; M-methanol extract, D- distilled water, Ac- acetone, C- chloroform, A- AgNps)

4.4.2. Minimum Inhibitory Concentration

Minimum inhibitory concentration was determined by the broth dilution method s aforementioned mentioned above in the methodology part. MIC values of methanol, distilled water, acetone and chloroform of crude extracts and AgNps synthesized from leaves extract of *V. sinaiticum* were summarized in **Table 5.** 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 Acetone ranged from 25 to 50mg/ml. Additionally, the MIC value of AgNps was 0.625mg/ml for both gram-negative (*E. coli* and *P. aeruginosa*) and *C. albican*. The result was given below in **Table 5.**

Table 5: MIC, MBC and MFC of crude extracts and AgNps

Extracts/ AgNps	Anti-microbial activity	Microbial strains				
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albican*
Methanol	MIC	12.5mg/ml	12.5mg/ml	25mg/ml	25mg/ml	12.5mg/ml
	MBC/MFC	50mg/ml	100mg/ml	100mg/ml	100mg/ml	100mg/ml
Water	MIC	25mg/ml	25mg/ml	50mg/ml	100mg/ml	100mg/ml
	MBC/MFC	50mg/ml	100mg/ml	100mg/ml	100mg/ml	Not detected
Acetone	MIC	25mg/ml	12.5mg/ml	50mg/ml	25mg/ml	25mg/ml
	MBC/MFC	100mg/ml	100mg/ml	100mg/ml	100mg/ml	100mg/ml
Chloroform	MIC	12.5mg/ml	25mg/ml	50mg/ml	25mg/ml	12.5mg/ml
	MBC/MFC	50mg/ml	100mg/ml	100mg/ml	100mg/ml	100mg/ml
AgNps	MIC	1.25mg/ml	1.25mg/ml	0.625mg/ml	0. 625mg/ml	0.625mg/ml
	MBC/MFC	5mg/ml	10mg/ml	5mg/ml	5mg/ml	5mg/ml

4.4.3. Minimum bactericidal and fungicidal concentration

The minimum bactericidal and fungicidal concentration were determined by subculturing 20µl contents of test tube containing MIC value and those test tubes follows/greater test tube of MIC values. The minimum fungicidal and bactericidal concentrations crude extracts and green synthesized silver nanoparticles leaf extracts of *V. sinaiticum* were summarized in **Table 5**. Methanol, distilled water and chloroform crude extracts were against all *S. aureus* and S. pyogenes, *E. coli*, *P. aeruginosa* cells at 50mg/ml and 100mg/ml MBC values respectively. Whereas, Acetone extract inhibited all bacterial cells at 100mg/ml MBC except *P. aeruginosa*/ 50 mg/ml/. In concerning of antifungal methanol, chloroform and acetone extracts were hampered all C. albican cells at 100mg/ml. Moreover, Green synthesized AgNps, displayed bactericidal and fungicidal activity at low concentrations when compared with crude extracts.

4.5. DPPH radical scavenging activity

DPPH assay is a rapid, sensitive, easy and inexpensive method to determine the antioxidant activity of plant extracts (Kedare & Singh, 2011). In this study 1ml of 0.004% methanolic DPPH solution was added into 3ml of prepared each series concentrations (50, 100, 150, 200, 250) µg/ml of methanol, distilled water, acetone, chloroform crude extracts and AgNps of *V. sinaiticum* leaf and Ascorbic acid as mentioned above. As a result, the discoloration of the DPPH from purple (pink) color to colorless/yellow produced the confirmation of DPPH radical scavenging by antioxidant present in the sample. Finally, the absorbance of each concentration was measured by using UV at 517nm and the results were given in **Table 6.**

The results given in the below table expressed that as the concentrations of each crude extract, AgNps and Ascorbic acid were increased, their absorbance values were reduced. This shows that increasing the amounts of concentration of samples (crude extracts, AgNps and standard ascorbic acid), maximizes the potential of the DPPH free radical scavenging activity by these samples. Therefore, the concentration and absorbance of the samples have inversely proportional. AgNps exhibited high scavenging activity that is comparable with the standard Ascorbic acid. Methanol extracts showed higher DPPH radical scavenging activity than Distilled water, acetone and chloroform. Next to methanol, chloroform extract exhibited the capability to discolorize DPPH free radicals more than distilled water and acetone extract. Acetone extracts displayed the least potential to discolorize free radicals as shown in **Table 6.**

Table 6: The absorbance of crude extracts, AgNps and standard

No	Conc (µg/ml)	The absorbance of samples/ extracts and Ascorbic acid standard					
		Methanol	DW	Acetone	Chloroform	AgNps	AA
1	50	0.308	0.322	0.395	0.314	0.108	0.067
2	100	0.206	0.249	0.320	0.238	0.081	0.062
3	150	0.183	0.229	0.302	0.210	0.082	0.063
4	200	0.179	0.194	0.286	0.177	0.068	0.054
5	250	0.146	0.175	0.178	0.154	0.067	0.050
	Absorbance of control (DPPH + methanol) = 0.550						

The percentage radical scavenging activity/ percentage of inhibition of all samples and standard ascorbic acid were calculated and given in **Table 7** and **Figure 8.** The result of the percentage of inhibition revealed that the samples possess a low percentage of radical scavenging activity

than ascorbic acid. The acetone extract exhibited the lowest (28.18- 67.72%) percentage of DPPH inhibition than others. Whereas, methanol extract presented with the highest percentage of free radical scavenging activity than other extracts which ranged from 44% to 73.45%. The variation in scavenging ability among the extracts was observed because of the ability of their phytochemical constituents to inhibit DPPH radicals.

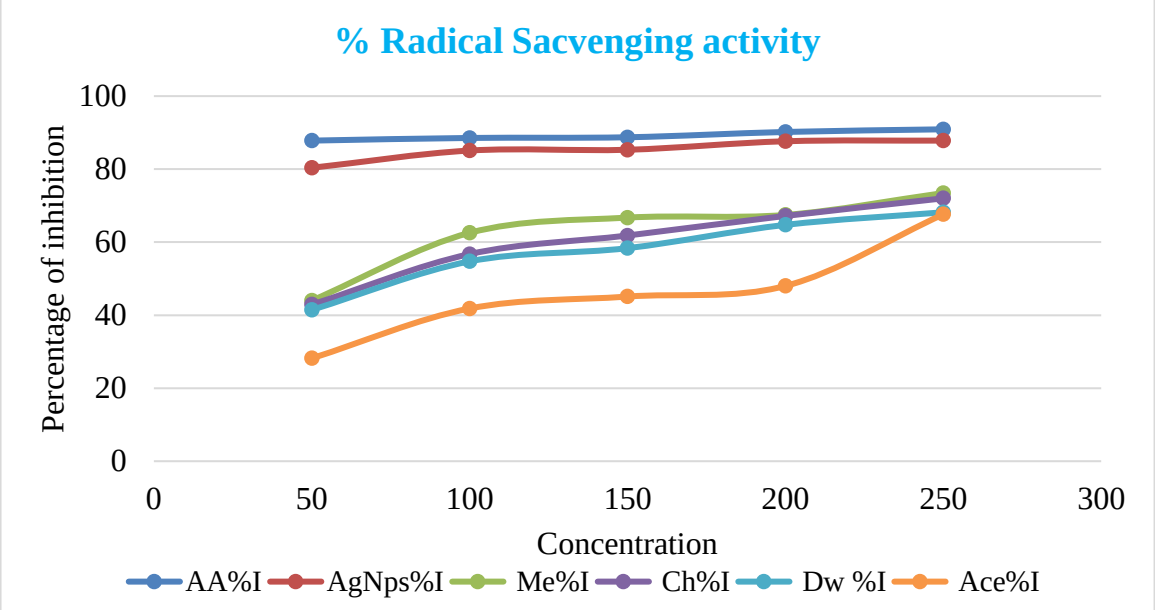

Figure 8: Calibration curve of percentage of inhibition of samples and ascorbic acid

The IC_{50} values of each sample/extract and AA were also calculated and summarized again in **Table 7.** As given in below table the IC_{50} value of acetone extract(172.798µg/ml) was greater than methanol, distilled water and chloroform. Whereas, the IC_{50} value of methanol extract was smaller (49.42µg/ml). Moreover, synthesized AgNps possess lower IC_{50} which is far away from the extract's values and comparable to the standard IC_{50} value. This finding showed that the extracts with low IC_{50} values have the potential to inhibit DPPH free radicals at low concentrations. Generally, this study revealed that *V. sinaiticum* plant displayed/manifested suitable antioxidant properties. Due to the presence of phenolics, terpenoids and flavonoids in the leaf part of this plant.

Table 7: Percentage of inhibition of DPPH radical and IC50 value of samples and AA

Con(µg/ml)	AA %I	AgNps %I	Methanol %I	Chloroform %I	Distilled water %I	Acetone %I
50	87.81	80.36	44	42.9	41.5	28.18
100	88.54	85.09	62.55	56.73	54.73	41.82
150	88.73	85.3	66.72	61.82	58.36	45.09
200	90.18	87.63	67.45	67.18	64.73	48
250	90.9	87.81	73.45	72	68.18	67.62
IC_{50} value	7.115	18.35	49.42	76.25	91	172.6
Linear regression equation	y=0.015x +49.886 R^2=0.9505	y=0.1098x+ 47.985 R^2=0.8412	y=0.1276x+ 43.694 R^2=0.8078	y=0.1373x+ 39.531 R^2=0.939	y=0.1269 x+38.452 R^2=0.9315	y=0.1701 x+20.624 R^2=0.8964

5. Discussion

The involvement of medicinal plants in different pharmacological roles has been reported in different parts of the world through scientific investigations. Since different parts medicinal plants contain numerous classes of bioactive compounds which are used as therapeutic agents to treat several human diseases (Riaz et al., 2023). This study revealed the qualitative phytochemical investigation of five solvents crude extract from the leaf part of *V. sinaiticum* **(Table 2)**. This qualitative study indicated the presence of tested phytochemicals including alkaloids, terpenoids, steroids, flavonoids, tannins, saponins, phenols and glycosides in the crude extract of *Verbascum sinaiticum*. Methanol solvents extract screened a wide range of phytochemical compounds than other solvents. Polar solvents have been screened a large number of bioactive compounds than non-polar solvents (Nawaz *et al*.,2020; Kaczorová *et al*., 2021).

This result of the current study was in line with other study conducted by Mergia and other authors demonstrated that methanol extract contained a large number of phytochemicals than aqueous extract (Mergia *et al*., 2016; Mergia *et al*., 2014). However, our study dis-agrees with this previously reported study since terpenoids and saponins were not detected in methanol extract. Due to the fact that phytochemical composition of plants is influenced by various factors like the method used for extraction procedures, geographical location, age of the plant, soil type, drying methods of plant material for experimental purpose, etc. (Renard *et al*., 2017; Ali & Endalew, 2021; Krakowska-Sieprawska *et al*., 2022). This study is also supported by other studies that 80% of methanol extract screened terpenoids, alkaloids, flavonoids and phenols from the leaf part of this plant (Lulseged *et al*., 2022).

Moreover, Yeabyo *et al*., (2018) have reported phenols, flavonoids, glycosides, saponins, alkaloids and steroids from the root part of this plant. The presence of these phytochemicals in this plant is the reason to have significant antimicrobial and antioxidant activity. Additionally, this study suggested that methanol solvent contained a higher yield of crude extract than other solvents used in this experimental work. Mergia *et al*., (2014) and Tadeg *et al*., (2005) reported

that methanol leaf extract of *V. sinaiticum* possess high yield than aqueous extract.

This current study also synthesized silver nanoparticles from the aqueous leaf extract of *V. sinaiticum*. The stirrer of reaction mixtures of plant extract and silver nitrate result in the color change from pale/greenish yellow into reddish/dark brown which indicated the formation of silver nanoparticles (**Figure 4**). The gradual color change of the mixture showed reducing of the Ag+ into silver nano particles by the action of capping agents of phytochemicals present in the plant extract (Ghramh *et al*., 2020; Jemal *et al*., 2017). Morphological characterization by SEM of this green synthesized nanoparticles exhibited mostly spherical shape with high agglomeration silver nano particles and distribution of various pores (**Figure 6**). As described by numerous literatures, the spherical shape of green synthesized AgNps frequently reported(Ahmed *et al*., 2016). Agglomeration of silver nanoparticles resulted from large amount of plant extracts and silver nitrate solution used during precursors preparation (silver nanoparticles synthesizing process). The silver nitrate to plant extract ratio affect the size and shape of green synthesized silver nanoparticles (Jalab *et al*., 2021). The high concentration plant extract maximized the chance of particles agglomeration (Kumar *et al*., 2017).The average size of green synthesized silver nanoparticles in this study was 47 to 80nm.

This study presented that different crude extracts of leaves of *V. sinaiticum* exhibited significant antimicrobial activity on tested microbial pathogen species including *S. aureus, S. pyogenes, E. coli, P. aeruginosa* and *C. albican* (**Table 4** and **Figure 7**)**.** All these crude extracts showed significantly showed high potential activity on gram-positive bacteria than on gram-negative bacteria. Since gram-negative bacteria are more resistant to antimicrobial agents due to the unique composition/structure of their outer membrane such as lipopolysaccharide and phospholipids which contribute them to survive harsh substances/environments by serving as physical barriers (Delcour, 2009; Heesterbeek *et a*l., 2019; Kleanthous & Armitage, 2015; Wink, 2015).

According to this study, methanol extract significantly exhibited higher antimicrobial activity than other extracts by showing a high inhibition zone that ranged from 17±1.0mm to

9.67±0.5mm *S. aureus* and *E. coli* respectively. This is due to the broad range of phytochemical constituents such as alkaloids, terpenoids, phenols, flavonoids, saponins, tannins and steroids of this crude extract. These phytochemicals can able to retard microbial growth by inactivating enzyme activity, inducing cell membrane disturbance/ anxiety, altering the permeability of cell membrane and cause bacterial cell lysis (Busani *et al*., 2012; Prosberg et al., 2016). Tadeg *et al*., (2005) reported that methanol leaf crude extract of *V. sinaiticum* against *S. aureus*(25±0.6mm) and *P. aeruginosa* (20± 0.5mm), while the zone of inhibition was not detected on *E. coli.* The finding of this study was supported by other investigations that demonstrated the antimicrobial activity of ethanolic leaf extract of this plant with their inhibition zone 20mm, 11mm and 12mm for *Enterococcus faecalis* (gram-positive*), E. coli,* and *pseudomonas aeruginosa/* gram-negative bacteria (Sener & Dulger, 2009).

Additionally, the crude extracts of the leaf of *V. sinaiticum* had significantly displayed antifungal activity by using the disc diffusion method (**Table 5** and **Figure 7**). Chloroform extracts showed a significant maximum zone of inhibition ranging from 13.0±1.0 to 8.0±1.0mm on *C. albican*. While distilled water extracts produced significantly smaller inhibition (11.0±1.0-7.33±0.5mm). This result was in line with studies conducted in Egypt reported as the ethanolic leaf extract of *V. sinaiticum* inhibited the growth of *C. albican* at range of 8 to 11mm zone inhibition (Ahmed *et al*., 2016). Moreover, our current study also agrees with results reported by Sener and Dulger found that the ethanolic leaf extract of this plant showed against *C. albicans* with 20 mm zone of inhibition (Sener & Dulger, 2009). However, this finding opposed the result previously reported by (Tadeg *et al*., 2005) in which the crude extract of the leaf part of this plant didn't produce zone of inhibition on *C. albican*.

Furthermore, this current study also presented the antimicrobial activity of green synthesized silver nanoparticles from aqueous leaf extract of the *V. sinaiticum* for the first time (**Table 4** and **Figure 7**). AgNps exhibited significantly higher inhibition zone/ antimicrobial activity/ than crude of plant extracts on tested microbes at very low concentrations. This results was in line with the studies of Mutairi *et al*., (2022) demonstrated that the AgNps derived from palm leaves extract shown higher antimicrobial activity than crude extracts of leaves of palm tree plant. A

number of studies have demonstrated that AgNps have the potential to hamper microbial growth through easily penetrating bacterial cell walls, forming free radicals on the surface of microbial membranes by releasing Ag ions, denaturation of protein synthesis by forming a bond with sulfhydryl group and destabilize DNA replication (Durán *et al.*, 2015; Ahmed *et al*., 2016; Srikar *et al*., 2016)

The antimicrobial activity of the leaf extract and green synthesized AgNps were also evaluated by determination MIC, MBC and MFC values as given in **Table 5**. From MIC values of crude extracts, methanol and chloroform extract were revealed lowest MIC value (12.5 to 25 mg/ml) against all tested microbial species. While distilled water extract possesses a high MIC value 100mg/ml against *C. albican*). As described by several kinds of literature, the different MIC values of the plant extracts were obtained because of their phytochemical composition variation and volatile nature (Mostafa *et al*., 2018). The MIC values of crude extract examined on bacterial strains were ranged 12.5 to 50mg/ml, While MBC values ranged 50 to 100mg/ml. The lowest MIC value was recorded for gram positive bacteria, whereas the highest values MIC value for gram negative bacteria. Because, gram-positive bacteria are more sensitive to antimicrobial agents than gram negative bacteria (Tadeg *et al*., 2005). In addition, chloroform and methanol extracts against *C. albican* with 12.5mg/ml and 100mg/ml MIC and MFC values respectively.

However, green synthesized AgNps displayed the highest antimicrobial activity with low MIC value (0.625 and 1.25mg/ml) for gram-negative bacteria, fungus and gram-positive bacteria respectively. Additionally, AgNps exhibited bactericidal and fungicidal activity with MBC and MFC values (5 to 10mg/ml). Gram-positive bacteria showed less susceptibility to AgNps, than gram-negative .This is due to the fact that the cell wall of gram-positive bacteria contains thicker negatively charged peptidoglycan which made difficult for nanoparticle/ silver ion to penetrate easily their cells (Ismail *et al*., 2016). AgNps have displayed antifungal activity, by rupturing and pore formation on the cell wall membrane of fungi that leads to death (Oves *et al.*, 2018). Finally, this study found that plant extracts exhibited higher antibacterial activity than antifungal

activity, because of the cell structural variety and organization between bacteria and fungi cells. Fungi are Eukaryotic possess well-organized and more complex cell structures than bacteria which made them effectively resist toxic substances as well as antimicrobial agents (Panáček *et al.*, 2009).

Several studies demonstrated that naturally occurring phytochemical compounds in medicinal plants have the potential to scavenge free radicals, due to their antioxidant properties (Yu *et al*., 2021). Our current study also evaluated the radical scavenging activity of different crude extracts and green synthesized AgNps of *V. sinaiticum* leaf extract by using DPPH assay and their IC_{50} was determined as given in the above (**Table 7** and **Figure 8).** According to the previous estimation of the level of antioxidant activity based on the IC_{50} values, this study revealed that methanol extract and AgNps displayed very high, chloroform and distilled water showed moderate and acetone extract possess less antioxidant value. Since when IC_{50} value is less than 50µg/ml, ranging from 50 to 100 and greater than 150µg/ml antioxidant properties is very strong, medium and low respectively (Molyneux, 2004; Saptarini & Wardati, 2020). The results of this study disagree with studies that reported high IC_{50} value (1.70mg/ml) in methanol extracts obtained from the leaf of *V. sinaiticum* (Lulseged *et al*., 2022). This variation may have come from the quality of plant material, the concentration used, the method used for extraction procedures, the drying method, etc.

In this current study high radical scavenging activity and low IC_{50} values of the leaf extracts of *V. sinaiticum* were determined due to the presence of various phytochemical compounds including phenols, terpenoids, glycosides and flavonoids in the leaf of this plant. Particularly, phenolics and flavonoids have a high potential to scavenge free radicals because of the arrangement of their functional groups, redox properties and number of hydroxyl groups which enhanced the antioxidant properties (Abeysinghe *et al*., 2021; Tungmunnithum *et al*., 2018).

Furthermore, green synthesized AgNps exhibited the highest percentage of radical scavenging activity (80.36-87.81%) than crude extracts (**Table 7**). This agrees with the study showed that

the antioxidant activity of leaf extract-derived AgNps(60.54%) was greater than aqueous leaf extract (44.49%) of *Ricinus communis* plant (Mintiwab & Jeyaramraja, 2021).In addition the IC_{50} values of AgNps were also lower than crude extracts(**Table 7)**. Khanal *et al*.,(2022) reported that the IC_{50} values(13.83µg/ml) green synthesized AgNps smaller than the IC_{50} values (15.86±4.14 µg/ml) of root extracts of *R. ellipticus* plant. This property of AgNps was shown because of the presence of an active functional group on the surface of this AgNps that originated in the leaf extract of this plant (Bhakya *et al*., 2016; Keshari *et al*., 2018). To the best of our knowledge, there is no report previously published on the evaluation of the antioxidant activity of green synthesized AgNps mediated by this plant extract.

6. Conclusion and Recommendations

6.1. Conclusion

The leaf crude extracts of *V. sinaiticum* were screened a wide range of phytochemical compounds. These phytochemicals contribute this plant to possess a significant biological activity and capability to synthesize silver nanoparticles. Each crude extract exhibited significant activity against both gram negative, gram-positive bacteria and fungi (*C. albican*). Methanol crude extract showed higher antimicrobial activity and followed by chloroform crude extract. The crude extracts also showed a high antioxidant activity. Additionally, Silver nanoparticles synthesized from leaf extract revealed higher antimicrobial and antioxidant activity than crude extracts. This suggests the extracts of this plant can be employed in the development of alternative novel drugs and used as antioxidants. Green synthesized silver nanoparticles from the aqueous leaf extract used as promises alternative antimicrobial agents and synthetic antioxidants.

6.2. Recommendations

This current study forwarded /recommended several points. Further study should be conducted on the antimicrobial activity of this plant on MDR microbes, isolation, characterization and purification of each class of bioactive compound from this plant, *in vivo* antimicrobial and antioxidant activity of *V. sinaiticum* should be conducted to confirms the *in vivo* antimicrobial and antioxidant activity of extracts. In addition, further studies should be conducted on other biological activity of leaf parts of this plant. This study also recommended, green synthesized silver nano particles should be optimized for various parameters and further characterized by other methods like UV, FTIR, TEM etc. Finally, future study should be needed to synthesize other noble metals such as gold, Zn, Cu etc. from leaf extract of this plant.

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