"*Moringa oleifera*: An Alternative to Synthetic Antimicrobials for Human Health"

A RESEARCH PROJECT

Submitted by

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UNDER THE GUIDANCE OF

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"Dissemination of education for Knowledge, Science and culture"

-Shikshanmaharshi Dr. Bapuji Salunkhe

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CERTIFICATE OF
RESEARCH PROJECT COMPLETION

This is to certify that **Miss. RACHANA TUKARAM PAWAR**studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled ***"Moringa oleifera*: An Alternative to Synthetic Antimicrobials for Human Health"** duringacademicyear2023-24.

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Place: Kolhapur

Date:

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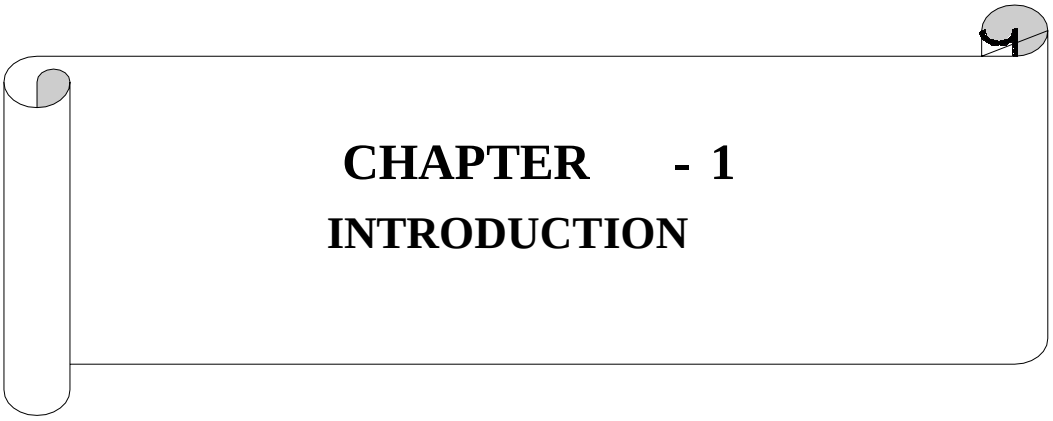


CHAPTER - 1

INTRODUCTION

INTRODUCTION

Nano technology is the branch of science which studies the fundamental principles of molecules and, deformation of materials by one atom or by one molecule. Nano materials are cornerstones of Nano science and nanotechnology. Nanostructure Science and technology is a broad and interdisciplinary area of research and development activity that has been growing explosively worldwide in the past few years. It has the potential for revolutionizing the ways in which materials and products are created and the range and nature of functionalities that can be accessed. It is already having a significant commercial impact, which will assuredly increase in the future.

The history of nano materials is too long. Nano materials were prepared and used in 19th Century. Decorative coloured glass windows which were used in old Churches and Palaces had used nanoparticles of iron, nickel, cobalt, silver, gold etc. Michael Faraday in 1857 has prepared stable colloidal solution of gold having particles of Nano size. (Singh M., Prasad S., Gambihr I. S. 2008) Photographic plates were covered by silver halide nano particles. But in those days due to lack of powerful microscope or other devices the size of the particle was remained unknown.

The progress of Nano science and nanotechnology took place in last three decades. In 1985 Curl, Kroto and Smalley synthesized sixty atoms carbon molecule later named it Fullerene. They were awarded 1996 Nobel Prize in Chemistry. In 1991, Iijima discovered carbon- nanotubes. In 2004, British Scientist Gem and Nevoselve discovered another allotrope of carbon named Graphene and for this discovery they were awarded 2010 Nobel Prize in Physics.

When dimension of material is reduced below 100 nm, then its mechanical, thermal, optical magnetic and other properties change than larger size properties of that material. Gold solution can be orange, purple green depending upon particle size. Due to smaller size larger surface area nano particles exhibit interesting and astonishing properties.

Moringa olifera is a plant which grows in tropical and subtropical region of south Africa. The common name of *Moringa olifera* is miracle tree because it has many numerous uses and adaptability. *Moringa olifera*, a native species of the Indian subcontinent, is a fast growing drought-resistant tree belonging to the family Moringaceae. It is widely cultivated for the diversified use of is younger Seed pod and green leaves as vegetables and for medicine. It is considered as very good supplement because of its high protein value. Without that, it is known as the miracle tree because of its diversified beneficial features, e., 10 times more vitamins than carrots 7 times more vitamin C than oranges, 17 times more calcium than milk and 15 times more potassium than banana (Rockwood J. L 2013). In addition, it helps to increase the blood antioxidant level (Kushwaha S et al., 2014) and reduce the blood sugar level. (C. William F., Lakshminarayanan S., Chegu H. 2012) and sustained inflammation (Libby P. 2002). The small leaves of moringa pack a full punch of nutrients which contain more protein than eggs, more iron than spinach. The moringa plant is found

as a good source of energy with potential as pharmaceuticals and cosmetics (oils from seeds for hair and skin care) benefits. Moringa seeds are also rich in vitamins and minerals. Seed extracts show antibacterial activity and are also used as water purifying agent various studies found Moringa seeds as oxidative stress- inflammation- blood sugar- and blood pressure- reducing agents. People suffering from malnutrition and poverty found moringa as a super food because of its nutritional alternative. Increasing demand in the dietary supplement and food applications.

Moringa olifera is a fast -growing tree that is classified as a vegetable that also serves as a medicinal plant. This miracle tree originates from the sub-Himalayan parts of India, and it can be grown in both tropical and subtropical region (magaji et.,al2020) and is able to withstand droughts and mild frosty weather, hence it can be cultivated anywhere in the world. This plant has gained medical and socioeconomic popularity because it has shown great health benefit and it is easy to cultivated. Traditionally, it is applied in diets to maintain healthy skin and it has also been used as a decoction to relieve stress and provide energy. All the parts of the plant can be utilized in a diet or as medicine since they are rich in minerals, proteins, vitamins, polyphenols, flavonoids, glucosinolates, isothiocyanats, alkaloid.3 For example, the leaves can be eaten raw, dried or taken as an infusion of an aqueous extract, while the bark is boiled in water or soaked in alcohol to make drinks and infusions that help with toothaches, stomach aches, the same is done to the roots. Furthermore, the leaves are utilized the most for medicinal purposes and they are a great source of prominent anti-inflammatory and antioxidants flavonoids, namely myricetin, quercetin and kaempferol. hypolipidemic, hypotensive and antidiabetic properties, antioxidants and anti-inflammatory. Other documented uses for these medicinal plants include its application as a diuretic, a testosterone stimulant antifungal and as an antibacterial. It can also be used to relieve a sore throat and symptoms of influenza or as anti-inflammatory agents. Interestingly, evidence has grown that Moringa oleifera contains hypoglycemic effects in diabetic animal models, including its associated complications such as oxidative stress and inflammation.

Moringa olifera plant is used for the cardiac circulatory tonic and antiseptic. The traditional use of *moringa olifera* leaves, the leaves are pounded up and used for the scrubbing utensils and for cleaning walls, seeds oil known as the ben oil is used for the delicate machinery. The oil is also useful for the manufacturing of perfumes. The non-governmental associations, the educational concerns for hungers organization, Church World service, and the trees for life have upheld the motto natural nutrition for the topics to animate the utilisation of several plant species groups as food sources, Including *Moringa olifera* has been found to process more than 90 nutritionally important chemical compounds namely, protein, lipid, vitamin minerals, carbohydrates and dietary fibers. Hence, it is used as dietary supplements in the topics as a nutrient source where malnutrition is a major concern especially in children and infants it is an outstanding indigenous source of vitamins, minerals and proteins. The aerial part of the plant (flower, young Shoots, leaves, immature pods) are a good source of essential amino acid methionine and are also rich in minerals such as phosphorus, calcium, potassium and iron. Plant seed are lipid-rich, having mainly stearic acid, palmitic acid, and oleic acid which represent about 30% of the dry weight. Seeds lipid composition is greater than the soyabean, making it nutritionally important and the refined seed oil acceptable. The substituted olive oil because of the presence of all the essential fatty acids in it. Legumes, poor in sulphur containing amino acid can be substituted by the seeds of *Moringa olifera*, Due to the higher level of essential amino acid. In addition, the higher nutritive contents present in dried leaves is an Indicator of the usefulness of the plant as a good resource to combat malnutrition.

Moringa olifera it useful in traditional medicines. Several pharmacological properties such as, anticancer, antimicrobial, antidiabetic, hepatoprotective and so on have already been claimed with experiments. In addition to this, plants have also been used to purify water by eliminating turbidity. Coagulant activity of seeds has also been employed to remove microbes from flocculated water as well as to remove heavy metals from the liquid solution. The high oleic acid content in the seeds oil

makes it an ideal candidate for cooking oil, cosmetic oil, biofuel, and also as a lubricant for machinery. Moringa stenopetala is traditionally being used to expel snakes.

Moringa has long been uses in herbal medicines by Indians and Africans. The presence of phytochemicals makes it a good medicinal agent. *Moringa olifera* can be used as an anticancer agent as it is natural, reliable and safe. The moringa can be used as an antineoproliferative agent, there by inhibiting the growth of cancerous cells. The compound of the leaves that are held responsible for the anticancer activities are glucosinolates, niazimicin has shown to be linked with cancer. previous reports have demonstrated that the *Moringa olifera* tree has anticancer properties. Cancer may occur in the body due to oxidative stress resulting from an imbalance between antioxidants and free radicals in the body. Therefore the great presences of antioxidants in *Moringa olifera* enable the plant to reduce oxidative stress, subsequently preventing cancer. Specifically, several metabolites found in *Moringa olifera* contribute to antioxidant properties, these includes saponins, tannins, beta carotene, flavonoids, phenolic acids and terpenoids demonstrated that boiled *Moringa olifera* pod had chemo preventive attributes, which reduced the occurrence and multiplicity of tumours in mice colon carcinogenesis induced by azoxymethane and dextran sodium sulphate. They attributed the inhibition of tumour cell proliferation to the presences of bioactive components water extracts of *Moringa olifera* had better anticancer activities when compared to hot water extracts, possibly because of heat inactivation of some bioactive molecules in the leaves. In addition, cold water leaf extract of *Moringa olifera* has been shown to be effective against cancerous cells of the lungs and liver of the mice evinced that a leaf extract of *Moringa olifera* caused 22% cell death and reduced proliferation of B16F10 murine melanoma cells. The authors observed 21.1% of apoptotic nuclei in the sub-G1 area and G2/M phase of the cell cycle. Concurrently there was an increase in p53 in the cells, which is a tumor suppressor protein. *Moringa olifera* together with plants from the family of brassicaceae, such as cabbage, broccoli and cauliflower contain relatively unique secondary metabolites compounds called glucosinolatin described glucosinolates as sulphur and nitrogen compounds

containing glycosidic secondary metabolites. These compounds include 4-(4'-O-acetyl-alpha-L-rhamnopyranosyloxy) benzyl isothiocynate, niazimicin, peterygospermin, benzyl isothiocynate, and 4(alpha-Lrhamnopyranosyloxy). Glucosinolates are found in all tissues of *Moringa olifera*.

Drugs sourced from medicinal plants helps to control diabetic progression in traditional settings. This medicinal plants are preferable to synthetic once for less toxicity and side effects it inhibits the pancreatic alpha amylase delay carbohydrates digestion causing a reduction in the rate of glucose absorption and lowering the postprandial serum level. *Moringa olifera* is a medicinal plant that has gained a lot of interest for its diverse biological properties. Reviewed evidence indicate the biological capability of this plant expand to protecting against complications linked with heart disease, cancer, fatty liver, and diabetes mellitus. For example, of previously published reviews supported the beneficial effects of the leaves of the *Moringa olifera* in improving blood glucose control in experimental models of diabetes. Recently Louisa and other supported the potential benefits of *Moringa olifera* cardiovascular or metabolic disorders, mainly by ameliorating the undesired proinflammatory response and inhibiting oxidative stress by mediating molecular mechanism such as hindering nuclear factor Kappa B (NF-kB) translocation or enhancing the antioxidant response of nuclear factor erythroid factor to related in different preclinical models. Thus, there is a need to better understand such as intracellular response of *Moringa olifera* setting of diabetes or in related metabolic complications the current study provide brief overview on *Moringa olifera* as medicinal plant followed by its therapeutic mechanism in controlling divers diabetic complications mostly focusing on understanding the modulatory effects of this medicinal plant in mechanism of inflammation and oxidative stress in a diabetic state.

Antioxidant are important substances that aid in eliminating oxidizing agents. Any imbalance of antioxidants caused by oxidative stress may lead to tissue damage. This may further prompt the disruption of lipids, membranes, nucleic acids and proteins which may further cause detrimental effect and metabolic complications. For years, the first line of drugs for metabolic complications

such as diabetes and related metabolic disorders have been metformin, thiazolidinediones and rosiglitazone but literature has proven that plant polyphenols and their bioactive compounds may potentially provide more efficiency in diabetes especially through targeting oxidative stress and inflammation to promote human health. For example, a studies on that moringa leaf and a has great scavenging activity as measured through the DPPH [1,1-diphenyl-2-picrylhaydrazyl (DPPH)-2,2-diphenyl-1picrylhydrazyl] and ABTS [2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid)]. However, these results were purely relevant to it's potential antioxidant potential, meaning that additional studies making use of established preclinical and clinical models of diabetes were still required to confirm the efficacy of this plant. Congruently, the below preclinical and clinical evidence discusses the rapeutic potential of this planter to reduce limit pathological features of oxidative stress and inflammation to alleviate complications linked with diabetes and related metabolic complications, without causing any adverse complications.

The term infection describes the growth of replication of micro-organisms in the host body. One of the alternative treatment for infections caused by bacteria is by using natural natural ingredients, such as *moringa olifera* leaves. The bacteria is Escherichia coli and staphylococcus aureus are responsible for the infection staphylococcus aureus are normal flora of human body present on the skin and mucous membrane. The Escherichia coli is responsible for the diarrhoea disease these bacteria are not pathogenic but normally present in the intestine. *Moringa olifera* leaves shows the anti-microbial activity against the Escherichia coli and staphylococcus aureus because the tannins and flavonoids are active compound in *Moringa olifera* leaves they inhibits the growth of bacteria. On the other hand, the use of for the control of various infections caused by microorganisms is well known, and in recent years scientific results have been generated that confirm it's antimicrobial activity. In vitro studies have been verified the activity of different parts of the plant in pathogenic microorganisms. Demonstrated the antifungal activity of essential oils of the leaves, and od alcoholic extracts of the seeds and leaves against dermatophytes. The main compound responsible

for the antimicrobial activity is benzyl 4-(4'o-acetyl-alpha-L-rhamnopyranosiloxy)-benzyl isothiocynate, which has bacterial action on several pathogenic species, including isolates of resistant to staphylococcus streptococcus and Legionella antibiotics. Due to the above, the objective of this study was to minimum inhibitory concentration of ethanolic extracts of *Moringa olifera* leaves on staphylococcus aureus and Escherichia coli.

Phytochemicals are the strictest sence of the word, chemicals produced by the plants. In particular, this plant family is rich in a fairly unique group containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates.

Different parts of the *Moringa olifera* tree have been established as being good sources of unique glucosinolates, flavonoids and phenolic acids carotenoids tecopherols polyunsaturated fatty acids (PUFAs) highly bioavailable minerals and folate Among Glucosinolates,4-0-(a-L-rhamnopyranosyloxy-) - benzylglucosinolat (glucomoringin)is the most predominant in the stem, leaves, flowers, pods and seeds of Moringa oleifera Although in the roots, benzyl glucosinolates (glucotropaeolin) is the most prominent. The highest content of glucosinolates is found in the leaves and seeds. The enzymatic catabolism of glucosinolates by the endogenous plant enzymes myrisinase produces isothiocyanats, nitriles, and thiocarbamates that are known for strong hypotensive (blood pressure lowering) and spasmolytic (muscle relaxant) effects. Among flavonoids, flavonol glycosides (glycosides, rutinosides, and malonyl glucosides) of quercetin>kaempferol>isorhamnetin are predominantly found in various parts of the tree, except in the roots and seeds. In the leaves the amount of quercetin and kaempferol was found to be in the range of 0.07-1.26 and 0.05-0.67%, respectively. Also, among different varieties, the Indian varieties [PKM-1 and PKM -2] have shown a higher total content of quercetin and kaempferol, compared to the African indigenous samples. The potent antioxidant activity M0 is attributed to the high concentration of these polyphenols. Of late, seven major cultivars of MO from Pakistan have been characterized for their polyphenolic, nutrient, and antioxidant potential. The quercetin apigenin, and kaempferol derivatives were recorded; the

major flavonoids in the hydromethanolic extract of the _Moringa_ foliage, corresponded to 47.0,20.9 and 30.0% of the total flavonoids (on an average), respectively. The varying concentrations of phenolics with the antioxidant capacity of the tested.

CHAPTER 2

AIMS AND OBJECTIVES

AIM AND OBJECTIVE

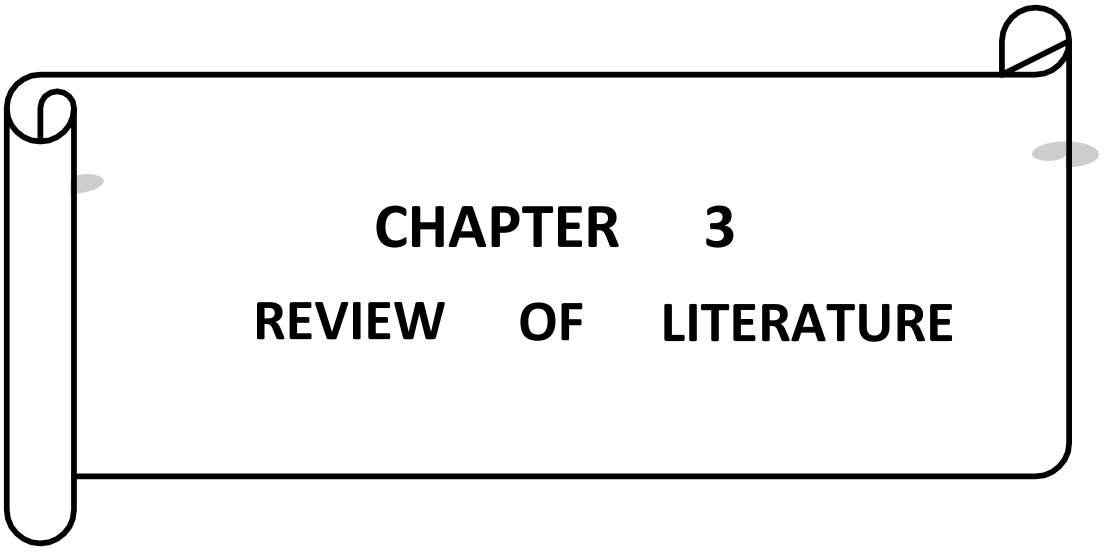
Aim - To study the antimicrobial activity and silver nanopartical synthesis from *Moringa olifera* .

Following objectives under taken for present study-

1) Determination of the antimicrobial activity of of *Moringa olifera olifera*

2) Phytochemical analysis of *Moringa olifera*

3) Antimicrobial activity of silver nanoparticles synthesizer from *Moringa olifera*



CHAPTER 3

REVIEW OF LITERATURE

Medicinal plants are playing major role in the treatment of many disease the extract of this plants can act as anti-inflammatory, antioxidant, anti allergic, anti cancerous, analgesic and antidiabetic due to this medicinal properties this plants have been used since century to cure and prevention of different kinds of disease. Derivatives from the medicinal plants and extracts are effective in small amounts, economical and safe to use, with negligible side effects morever medicinal plants are easily accessible and have better compatibility

Moringa olifera, commonly known as drumstick tree or horseradish tree and grows in the tropical and subtropical regions of the World and belonging to the family of moringace. Availability of different essential phytochemicals in leaves and seeds of *moringa olifera* contains high nutritional values. *M. olifera* leaves have the high content of proteins has ideal levels of essential amino acids such as methionine, cysteine, tryptophan and lysine. *Moringa olifera* has a number of uses such as for human consumption and is a plant rich in antioxidant compounds which are of great importance in preventing trees causing several degenerative disease. A number of medicinal properties have been referred to various properties of this esteemed tree. The leaves, roots, barks, seeds and seed oil have been used for alimenta, in South Asia, including the treatment of inflammation and infectious disease along with cardiovascular, gastrointestinal and heamatological diseases

There is an increase in natural product usage towards fulfilling its role of treating and preventing diseases in humans. Therefore, drugs with natural sources have higher efficacy and are a drug of choice than synthetic ones (Kadir et al., 2013). *Moringa oleifera*, commonly known as horseradish tree, is a pan-tropical species of large-sized trees sourced from the sub-Himalayan regions in North India, Bangladesh, Pakistan and Afghanistan. Other common names are moringa, benzoil tree, drumstick tree or horseradish (Fahey, 2005). Most parts of *M. oleifera*, namely seeds, leaves and pods, were used as components in traditional medicine (Abdull Razis et al., 2014, Gopalakrishnan et al., 2016). The leaves and pods were commonly consumed in India and South Africa (Ahmad et al., 2018). Then, parts of *Moringa oleifera* are examined for medicinal properties associated with different bioactive ingredients, such as phenolic acids, vitamins, isothiocyanates,

flavonoids, saponins, and tannins that exist as essential quantities of ingredients in a plant. Leaves of Moring oleifera are used in different types of chronic diseases, including dyslipidemia, hypertension, diabetes mellitus, fatty liver, malignancy, pain and fever reduction, asthma, and inflammation (Martín et al., 2013).: the *moringa olifera* tree has anticancer properties. Cancer may occur in the body due to oxidative stress resulting from an imbalance between antioxidants and free radicals in the body. Therefore the great presences of antioxidants in *Moringa olifera* enables the plant to reduce oxidative stress, subsequently preventing cancer. Specifically, several metabolites found in *Moringa olifera* contribute to antioxidant properties, these includes saponins, tannins, beta carotene, flavonoids, phenolic acids and terpenoids demonstrated that boiled *moringa olifera* pod had chemo preventive attributes, which reduced the occurrence and multiplicity of tumours in mice colon carcinogenesis induced by azoxymethane and dextran sodium sulphate. The compounds include 4-(4'-O-acetyl-alpha-L-rhamnopyranosyloxy) benzyl isothiocynate, niazimicin, peterygospermin, benzyl isothiocynate, and 4(alpha-Lrhamnopyranosyloxy). Glucosinolates are found in all tissues of *moringa olifera*.

Diabetes mellitus (DM) is a slow damaging disease known worldwide due to the low insulin production or created damaged insulin that is unusable for body cells. Three types of diabetes are labelled as gestational diabetes, type-1 and type-2 diabetes. Type-1 diabetes mellitus causes autoimmune damages responsible for insulin production, especially beta cells in the pancreas. Type-2 diabetes mellitus leads to inefficient in insulin production. Gestational diabetes occurs in pregnant women with no diabetic history, where high blood sugar level happens throughout pregnancy (Siddiqui et al., 2013). Drugs sourced from medicinal plants help to control diabetic progression in traditional settings. These traditional medicinal plants are preferable to synthetic ones for less toxicity and side effects (Yuan et al., 2016). Degradation of the dietary starch proceeds rapidly and leads to elevated PPHG (postprandial hyperglycemia). It has been shown that activity of HPA (human pancreatic α-amylase) in the small intestine correlates to an increase in postprandial glucose

levels, the control of which is therefore an important aspect in treatment of type-2 diabetes (Khajaria et al., 2013) Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the postprandial serum glucose levels. In 1970 s, it was realized that inhibition of all or some of the intestinal disaccharidases and pancreatic α-amylase by inhibitors could regulate the absorption of carbohydrate and these inhibitors could be used therapeutically in the oral treatment of the noninsulin-dependent diabetes mellitus ie., type-2 diabetes (Vijan et al.,1997).Recently Louisa and other supported the potential benefits of *moringa olifera*_ cardiovascular or metabolic disorders, mainly by ameliorating the undesired proinflammatory response and inhibiting oxidative stress by mediating molecular mechanism such as hindering nuclear factor Kappa B (NF-kB) translocation or enhancing the antioxidant response of nuclear factor erythroid factor to related in different preclinical models. Thus, there is a need to better understand such as intracellular response of *_moringa olifera* setting of diabetes or in related metabolic complications the current study provide brief overview on *moringa olifera* as medicinal plant followed by its therapeutic mechanism in controlling divers diabetic complications mostly focusing on understanding the modulatory effects of this medicinal plant in mechanism of inflammation and oxidative stress in a diabetic state.

Free radical and reactive oxygen species are well known as inducers of cellular and tissue pathogenesis which is causing some diseases like diabetes, cancer, inflammatory and also cardiovascular. Free radical reactions take place in the human body and food systems can causing injury and death (Halliwell,B.,2008). Free radicals are one of the main factors which necessary to cause DNA mutation, which is involve in the initiation stage of carcinogenesis (Johnson et.,2007). Reactive Oxygen Species (ROS) are constantly produced in human body by normal metabolic system. An over-production of reactive oxygen can occur the imbalance of defense system. Therefore, investigations of antioxidants are needed which focused on natural compounds from natural sources Most of the antioxidant compounds derived from plant source have wide variety and

chemical properties. The antioxidant characteristic is based on its ability to trap free radicals. *Moringa oleifera* (Moringaceae; Indonesian name: kelor) is an ornamental plant native in tropical and subtropical areas, and commonly cultivated in all region of Indonesia as a vegetable for cooking purposes. . *M. oleifera* leaves contain of natural source of polyphenol that potential to have antioxidant. The purpose of this paper is to evaluate the antioxidant activity of various extract of *M. oleifera* leaves.

The biological synthesis method is safer, more useful, and less expensive. Also, there are many metals used such as gold, silver, lead, zinc oxide, copper, and others (Ying S et al. 2022), and silver nanoparticles are the most promising. It has been observed that silver nanoparticles do not affect living cells, so they cannot provoke microbial resistance (Singh M, et al.,2008) and silver nanoparticles have strong antimicrobial activity (Karani et.al2013). Medical plants have occupied an essential role in the lives of people all over the world, starting with ancient Indian Ayurvedic and traditional medicine. The plant phytochemicals or secondary metabolites such as proteins, polyphenols, phenolic acids, ketones, terpenoids, and amides constantly play a significant role in the biosynthesis of nanoparticles (Salayon A et al.,2021) Moringa contains 13 species and is called by many common names according to the growing regions. Moringa has been used in the biosynthesis of nanoparticles by a number of researchers (Vibhute S et al.,2014). the ecologically friendly manufacture of silver nanoparticles from fresh leaves of the Moringa oleifera plant and evaluated against *Escherichia col*i, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Bacillus subtili*s. Moringa contains a long list of medical use including antibacterial(Bijal A et al.,2015), antifungal Aondo T et al.,2018), antiparasitic (Hefazi A et al.,2018), antiviral(Mohamed A et al.,2017) , wound healing, antimicrobial, antioxidant, and proliferative properties (Al-Ghanayan A et al.,2022)

Infection disease is the most suffered disease by the population of developing countries including Indonesia. One of the causes is bacteria . The term infection describes the growth of replication of microorganism in the host's body. The disease grows when the infection produces changes to the

normal body physiology (Sylvia T.Pratiwi 2008) One of the alternative treatments for infections caused by bacteria is by using natural ingredients, such as Moringa oleifera L. plants. This plant is referred to as the world's most valuable multipurpose and miracle tree because all parts of the plant are useful for food, medicine, cosmetics, or water purified (Small E 2012). Moringa oleifera L. leaf pharmalogically has benefits as antimicrobial, antifungal, antihypertensive, antihyperglycemic, antitumor, anticancer, anti-inflammatory (Deyno.S T A 2014). One of the most prominent effect is antioxidants. Based on phytochemical tests, Moringa oleifera L. leaves contain tannins, steroids, triterpenoids, flavonoids, saponins, interquinones, and alkaloids that all are antioxidants (Kasolo J N 2010). Based on a research by Fuglie, fresh moringa leaves has antioxidant strength seven times higher than vitamin C (Fuglie Lowell J 2001). One of the flavonoid group that moringa has, is quercetin. It contains antioxidant 4 to 5 times higher than vitamin C and vitamin E (Vergara-Jimenez M. et.,al 2017). It is known that tannin and flavonoids are active compounds in Moringa oleifera L. moringa leaf extract with ethanol solvent are able to inhibit the formation of S. aureus, and have antibacterial properties.

Antimicrobial resistance (AMR) has emerged as a global threat to the current healthcare system that might impede the control of many infectious illnesses and significantly regress modern medicine. The overuse and abuse of antimicrobials in medical and non-medical settings have exacerbated the global epidemic of AMR since the discovery of the first antibiotics that consistently benefitted human medicine. Due to the risk of the emergence of resistance, even novel antimicrobials that aim to inhibit bacterial growth cannot be trusted for sustained administration. Antimicrobials with new modes of action against MDR pathogens are still in the early stages of development (Qais FA. et al.,2020) and are being actively influenced by research in the realm of nanotechnology. By reducing the material to a nanoscale, the characteris-tics can be enhanced, improving function (Bindhu MR.et al.,2020). The physi- cal and chemical strategies for nanoparticle synthesis might result in unforeseen consequences like high energy use and environmental

contamination (Gupta R,Xie H 2018). The “green synthesis” approaches/methods are receiving much interest in the pre- sent research because they decrease waste generation, reduce pollution, and employ cleaner, renewable auxiliary materi-als. Hence, the green production of nanoparticles is like-wise rated as an environmentally benign method (Qais FA.et al.,2020). It has been determined that the green synthesis of nanoparticles, which uses plant extracts and microorganisms for synthesis,is a more environmentally friendly method for synthesizing AgNPs. Polyphenols, proteins, amides, phenolic acids, ter-penoids, and ketones are examples of plant phytochemicals or secondary metabolites that continuously play a crucial role in the creation of nanoparticles (Salayova A.et al.,2021). Many researchers describe the production of AgNPs with substantial anti- bacterial activity using plant extracts: Murraya koenigii (curry leaf)(Christensen L.et al.,2021), Xanthium strumerium L. (Mittal J.et al.,2017), Macrotyloma uniforum (Vindhu J.et al.,2011), root extracts of Trianthema decandra (Geethalakshmi R.et al.,2012). The antibacterial efcacy and stability of AgNPs is depend ent on the propensity to form aggregates and the ability of releasing silver ions (Panacek A et al.,2006). AgNPs coated with Agrimoniae herba extract demonstrated both as reducing and stabilizing agents, which enhanced therapeutic efficacy (Qu D.et al.,2014). The primary metabolites found in plant extracts, such as reducing sugar, proteins, peptides, amino acids, etc., are crucial for the reduction and stabilization of metallic silver into AgNPs.The synthesis of stable AgNPs is brought about by favonoids and terpenoids found in the leaf extract of A. Indica (Shankar SS.et al.,2006). Numerous studies revealed that proteins may also serve as stabilizing agents in addition to bioreduction (Reddy M.et al.,2015). With the increasing problem of multi-drug resistance in patho-genic bacteria, the efcacy of available antibiotics and otherantibacterial agents is continuously decreasing. To combat such pathogenic bacteria, novel anti-infective drug targets such as quorum sensing and bioflms seem to be attractive options. In recent years, green synthesized nanoparticles have demonstrated activity against QS-mediated virulence factors in pathogenic bacteria. However, research data on broad-spectrum green synthesized active nanoparticles with improved efcacy and stability is limited. Therefore, we hypothesized that green synthesis of silver nanoparticles using

bioactive plant extract might result in the development of silver nanoparticles with improved efcacy and stability against bacterial pathogens. Hence, this work used Moringa oleifera leaf extract (MOL) to synthesize AgNPs.

CHAPTER 4

MATERIALS AND METHODS

1.Material and methods

1.1 Materials

Reagents –

1. Mayer's reagent

Composition: Mercuric chloride - 1.36 gm

Potassium iodine - 5 gm

D/W - 100ml

2. Benedict's reagent

Composition:

Copper sulfate pentahydrate - 17.3 gm

(CuSO4.5H2O)

Sodium carbonate (Na2CO3) - 100 gm

Sodium citrate. - 173 gm

Distilled water - 1000 ml

3. Biuret reagent

Composition: Copper sulphate - 1.5 gm

Potassium sodium tartrate - 6 gm

2M sodium hydroxide. - 375ml

Potassium iodine - 1 gm

D / W. - 500ml

Media

4. Nutrient agar

Composition :

Beef extract - 1 gm

Yeast extract - 2gm

Peptone - 5 gm

Sodium chloride - 5 gm

(Nacl)

Agar - 15 gm

2.1 Method

Sample collection-

Moringa leaves is directly collected from farm. The leaves were cleaned with tap water three to four times before detached from the shoots. Then leaves are dried in sun light and in shade . Later they grind in blender to form fine powder.

***Moringa oleifera*leaf extract preparation –**

For extract preparation 10gm fine powder of *M. oleifera* leaves (sun \shade) dried were added into 300 ml different solvent and keep the flask overnight in the shaker. Three different solvent i.e. methanol ,ethanol and chloroform were used for extract preparation. The obtained extract was filtered using filter paper and stored at 4°for further use

Antimicrobial action of extract-

The antimicrobial effect of the extract was determined by agar diffusion method .The tests were evaluated against micro-organisms including the *Escheria coli, Staphylococcus aureus. Pseudomonas aeruginosa* . In agar diffusion method , suspension of test organism was spread on sterile plates of nutrient agar and 100 µL sample solution i.e plant extract was added in agar wells , after addition of the sample plates were kept in refrigerator for 30min for diffusion and incubated at 30°c for 24hrs . After incubation plates were observed for zones of inhibition, which appear as a clear area around the wells. The diameter of zones of inhibition was measured and recorded .

Phytochemical screening of M. oleifera leaves extract :

Qualitative analysis:

Phytochemical screening of the *M. oleifera* extract was conducting using qualitative chemical test for the identification of various phytochemical constituents such as Alkaloids, Saponins, Tannin, Carbohydrates, Reducing sugar and protein by using standard procedure.

1. Test for Alkaloids

1ml of extract was treated with Mayer’s reagent (Freshly Prepared). Development of yellow or cream coloured PPT shows presence of alkaloids.

Mayer's reagent -

Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml)

2. Test for Carbohydrates

1ml of extract was treated with alcoholic alpha – Naphthol solution (shake well) followed by addition of concentrated sulphuric acid. Development of violet colour at the junction of two liquid shows presence of carbohydrate.

3. Test for reducing sugar

1ml of extract with 1ml of Benedict’s reagent was taken in test tube. Keep in boiling water bath for 5min. Orange red PPT shows presence of reducing sugar.

Benedict's reagent -

One litre of Benedict's reagent can be prepared by mixing 17.3 grams of copper sulfate pentahydrate (CuSO4.5H2O), 100 grams of sodium carbonate (Na2CO3), and 173 grams of sodium citrate in distilled water.

4.Test for Saponins

1ml of extract shaken with 2 ml of distilled water. Persistent of foam produced for 10 min shows presence of saponins.

5. Test for Tannins

1ml of extract was treated with 2-3 drops of ferric chloride solution. Development of green colour shows presence of tannins.

6. Test for proteins

1ml extract was treated with biuret reagent. Formation of pink (purplish violet) colour shows presence of proteins.

Biuret reagent -

Dissolve 1.5 g copper (11) sulphate-5-water crystals, 6 g potassium sodium tartrate-4-water in 500 ml of distilled water. Add 375 ml of a 2M sodium hydroxide solution while stirring. If a precipitate occurs add 1 g of potassium iodide.

***M. oleifera* leaf extract preparation for nanoparticles synthesis :**

For extract preparation 10gm fine powder of *M. oleifera* leaves was added into 300 ml D/W. Then it was heated until the extract was ½ to ¾ at 100%c. The obtain extract was filtered using filter paper.

Precursor preparation :

Silver nitrate (AgNo3) was used as precursor for the synthesis of silver nanoparticles from the *M. oleifera* leaves extract .0.01mM solution of silver nitrate was prepared.

Biosynthesis of silver nanoparticles :

The green synthesis method was followed for the synthesis of silver nanoparticles for the reduction of Ag+ ions.

10ml of 0.01 mM aqueous solution of AgNo3 was added dropwise into 10 ml *M. oleifera* leaves extract and mix it properly by using a magnetic stirrer. Later the change in colour was observed from light brown to dark brown which indicated the formation of silver nanoparticles.(P.Singh et al.,2017

Antimicrobial action of Silver nanoparticles :

The Silver nanoparticles synthesis using *M. oleifera* leaves extract were tested for their antipathogenic activity against pathogenic fungi. The antimicrobial effect of the nanoparticles was determined by agar diffusion method .The tests were evaluated against micro-organisms including the *Escheria coli, Staphylococcus aureus. Pseudomonas aeruginosa and Candida albicanc* . In agar diffusion method , suspension of test organism was spread on sterile plates of nutrient agar and 100 µL sample solution i.e silver nano partical was added in agar wells , after addition of the sample plates were kept in refrigerator for 30min for diffusion and incubated at 37°c for 24hrs . After incubation plates were observed for zones of inhibition, which appear as a clear area around the wells. The diameter of zones of inhibition was measured and recorded.

CHAPTER5

RESULTS AND DISCUSSION

Result and discussion

1) Antimicrobial action of plant extract:

M. oleifera leaves extract was prepared after washing, dried and grinding leaves into powder form. The powder was mixed with different solvent such as Ethanol, Methanol, Chloroform and Aqueous solution. Test organisms are spread on sterile solidify nutrient agar plate by using sterile glass spreader. After spreading of suspention on sterile solidify nutrient agar plate and addition of plant extract in agar well plates were kept for diffusion in the refrigerator for 30 min. and incubated at 37°C for 24 hrs. After incubation plates were observed for clear zone of inhibition.

The *Moringa oleifera* plant extract showed inhibition zone against *Escherichia coli* (Gram negative) and *Sataphylococcus aureus* (Gram positive) bacteria.

Sr. No	Organisms / Extract	Control		Sun dried		Shade dried	
		S.aureus	E.coli	S.aureus	E.coli	S.aureus	E.coli
1.	Methanol	13mm	12mm	13mm	17mm	18mm	10mm
2.	Ethanol	11mm	12mm	18mm	18mm	18mm	16mm
3.	Chloroform	-	-	13mm	14mm	12mm	13mm
4.	Aqueous	-	-	17mm	22mm	11mm	16mm

Fig.1 : Antimicrobial activity of M.oleifera plant extract

Antimicrobial activity against different extract :

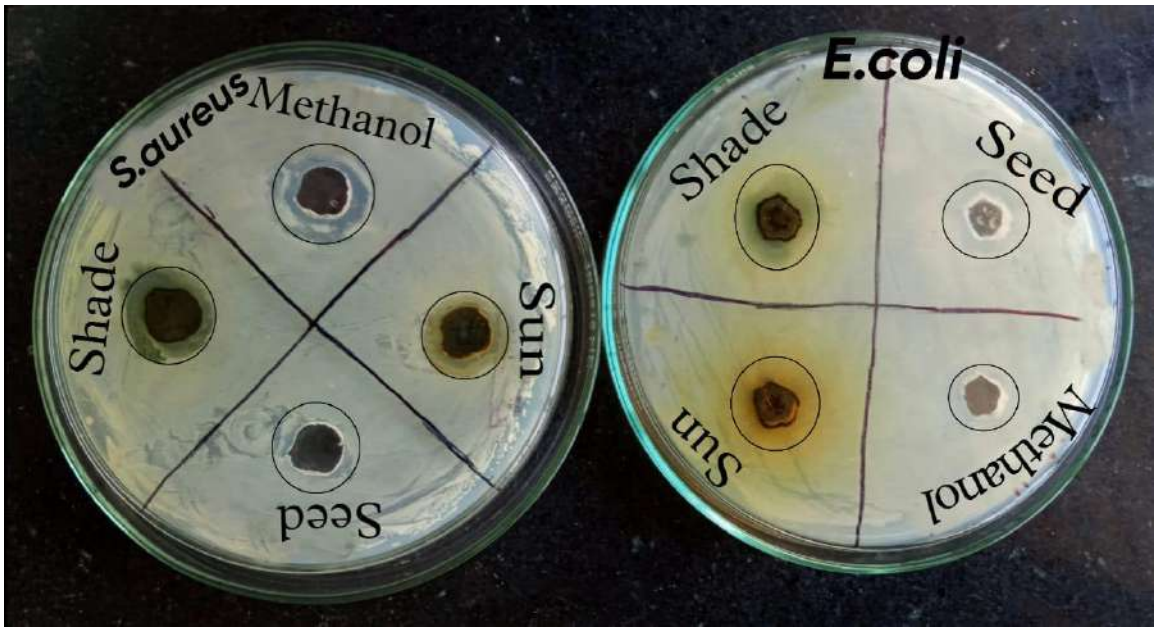


Fig. Inhibion zone of Methanol extract

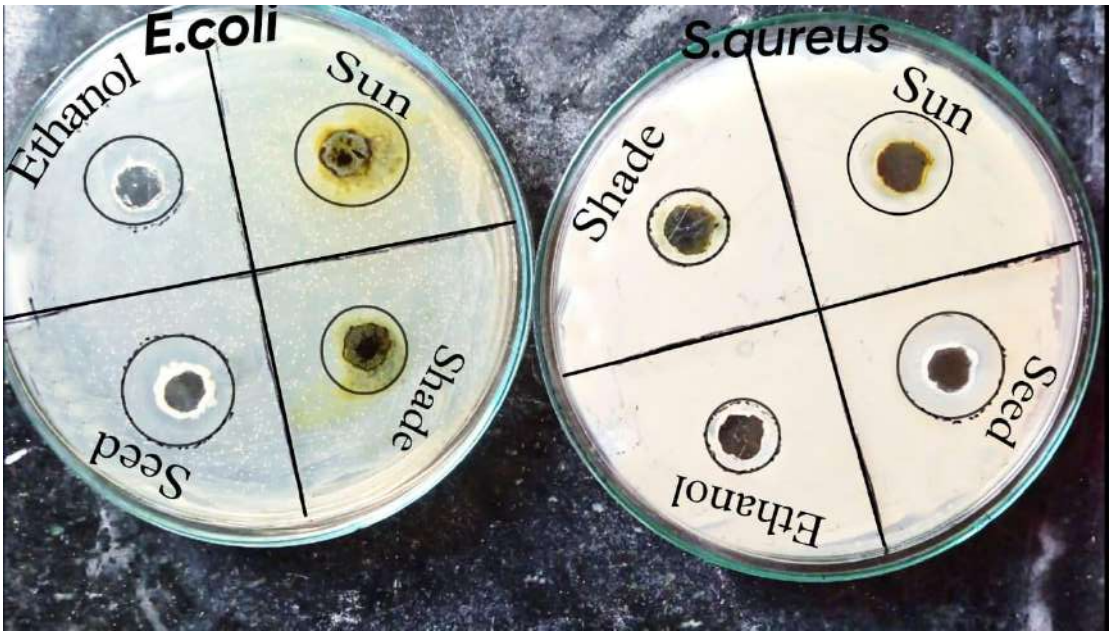


Fig. Inhibition zone of Ethanol extract

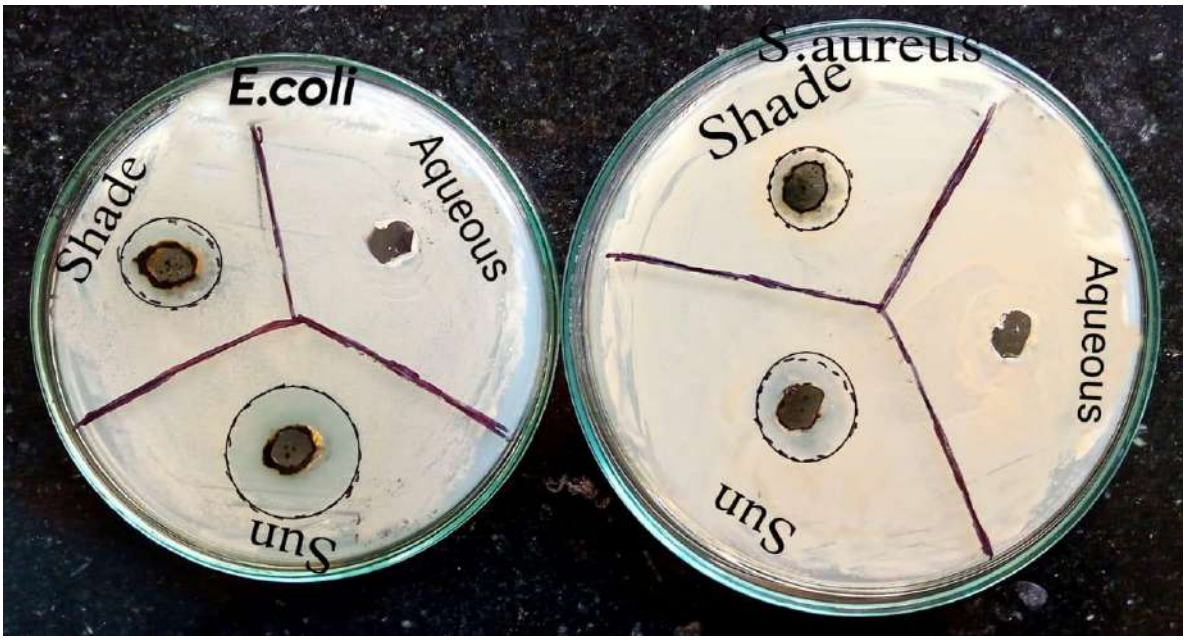


Fig. Inhibition zone of Aqueous extract

Fig. Inhibition zone of Chloroform extract

2) Phytochemical analysis of *M. oleifera* :

The aqueous extract (Sun and shade dried) of the *M. oleifera* were qualitatively analysed to determine various phytochemical compounds in the plant. The common phytochemicals from plant such as alkaloids, saponins, tannins, carbohydrates, reducing sugar and protein were tested.

Fig. Sun dried **Fig. Shade dried**

Fig. Alkaloids test -

Sun dried - Positive; Shade dried - Positive

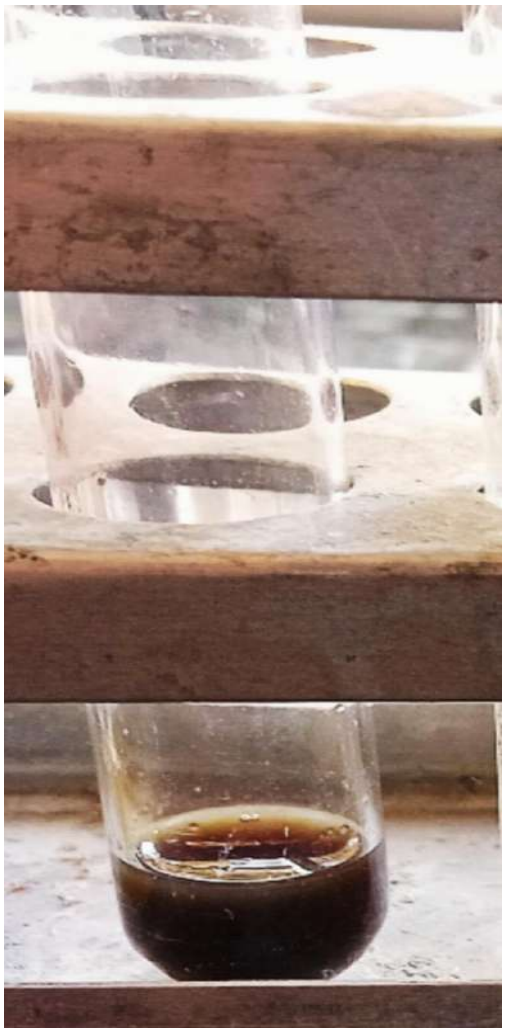


Fig. Sun dried. **Fig. Shade dried**

Fig.Carbohydrates test -

Sun dried - Positive; Shade dried - Positive

Fig. Sun dried. **Fig. Shade dried**

Fig . Test for Reducing sugar -

Sun dried - Positive; Shade dried - Positive

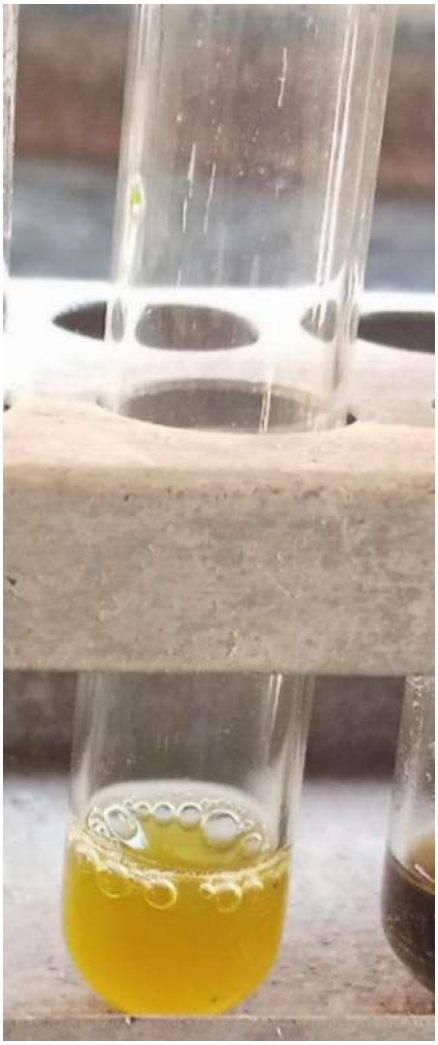


Fig. Sun dried	Fig. Shade dried

Fig. Test for Saponins -

Sun dried - Positive; Shade dried - Negative

Fig. Sun dried	Fig. Shade dried

Fig. Test for Tannins -

Sun dried - Positive; Shade dried - Positive

Fig. Sun dried	Fig .Shade dried

Fig . Test for protein -

Sun dried - Negative; Shade dried - Negative

Standard result for phytochemical analysis:

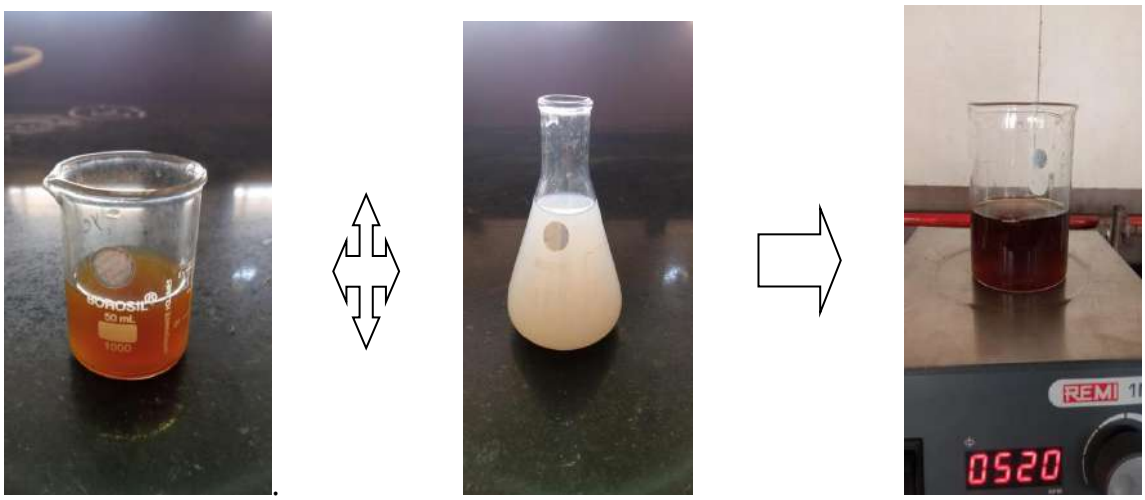
Sr.No.	Constituents	Method	Result	
			Sun dried	Shade dried
1.	Alkaloids	Mayer's test	+	+
2.	Carbohydrates	Molish' test	+	+
3.	Reducing sugar	Benedict's test	+	+
4.	Saponins	Foam test	+	-
5.	Tannins	Ferric chloride solution add to aqueous extract – greenish black colour developed	+	+
6.	Protein	Biuret test	-	-

+ - Positive result

- - Negative result

3) Antimicrobial action of Silver nanoparticles :

Moringa olifera leaves extract was prepare after washing, drying and grinded leaves into powder form the powder was mixed with D/W. Then it was heated until the extract was 3/4. The obtain extract was filtered using filter paper. AgNo3 was mixed with plant extract to form MLE AgNPs. The main sign of silver nanoparticle formation was the change in colour from light brown to dark brown. MLE works as a reducing agent as well as capping agents. Under the necessary conditions such as constant stirring and temperature, free silver atoms agglomerated and provided the basis for the formation of colloidal silver nanoparticles.



Extract **a) AgNP3** **b) AgNPs**

Fig. Synthesis of silver nanoparticles using *M. oleifera* leaves extract.

Sr.No.	Organisms	Inhibition zone of nanoparticles (mm)	
		Low conc.(50 µL)	High con. (100µL)
1.	*S.aureus*	12 mm	14 mm
2.	*E.coli*	-	-
3.	*C.albicus*	10 mm	11 mm
4.	*Protease* spp.	12 mm	13 mm
5.	*Pseudomonas* spp.	16 mm	16 mm
6.	*S.typhi*	13 mm	15 mm
7.	*Klebsiella* spp.	11 mm	12 mm

Fig. Effect of silver nanopartical (AgNPs) biosynthesized from *M. oleifera* extract against bacterial and fungi pathogen.

The Antimicrobial activity assay against some pathogens fungi and different Gram negative and gram positive bacteria using the well agar diffusion methods.

Fig. Staphylococcus aureus

Fig. Escherichia coli

Fig. Candida albicus

Fig. *Protease* spp

Fig . *Pseudomonas* spp.

Fig. *Salmonella typhi*

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Fig. *Klebsiella* spp.

CHAPTER 5

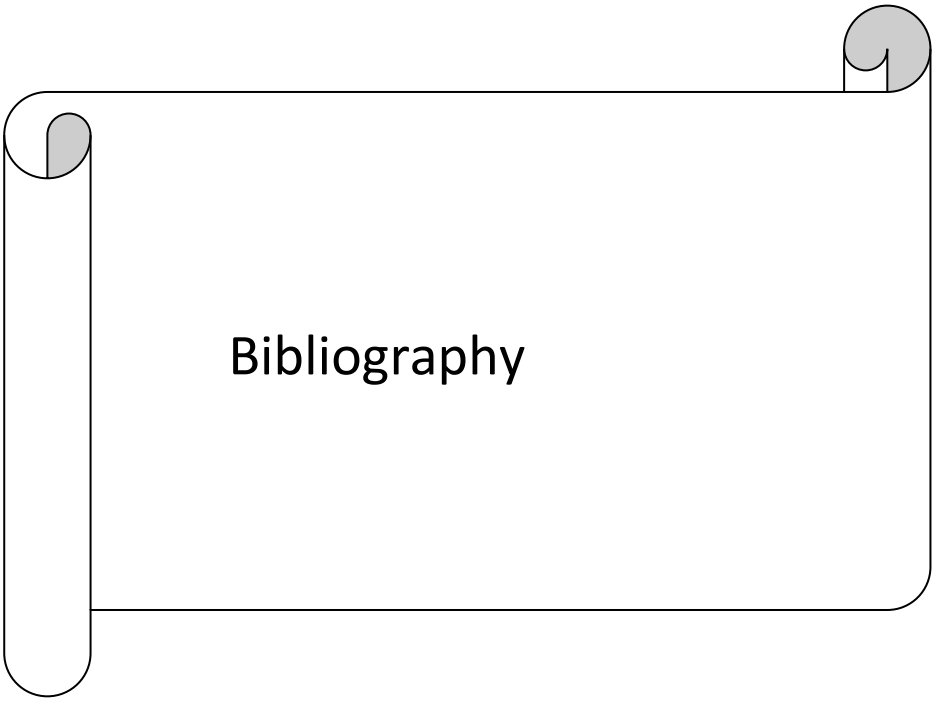
CONCLUSION

Conclusion:

Moringa oleifera leaves extract has antimicrobial activity against different pathogenic organisms. from this result we can conclude that extract has potential to use in various organic preparation.

From the phytochemical analysis of extract it is clear that the aqueous extract is rich in alkaloids, saponins and tannins. Therefore we can use this extract as reductant in silver nanoparticles synthesis by green synthesis method.

The synthesized silver nanoparticles were operated as an active substrate against different pathogenic bacteria and fungi which is recognized resistant against maximum commercially available drugs. This green synthesis of silver nanoparticles may prove a quick, cost free and suitable alternative of synthetic antibiotics against multi drugs resistance bacteria. Conclusively, *M. oleifera* leaves extract produce AgNPs with good stability power in the solution and broad antibacterial activity.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