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Green synthesis of silver nanoparticles using medicinal plant *Moringa oleifera* leaf extract and assessment of its antioxidant activity

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Abstract
Nanotechnology is most active research area in the modern material science. Synthesis of silver nanoparticles (AgNPs) using plant green leaf extract is achieving popularity because of its environmental friendly nature and cost effective and also use of aqueous extract of leaves is for stabilization or reduction purpose. Nowadays, silver nanoparticles (AgNPs) are attracting attention due to their attractive properties, such as the good electrical and thermal conductivity, photo-electrochemical activity, chemical stability and high catalytic and antimicrobial and antioxidant activities. The characterization of silver nanoparticles, can be done by Uv-Vis spectrophotometer, whereas they can be detected by microscopic technique like TEM, SEM, XRD, FITR etc. The antimicrobial activity can be done with bioassay technique by employing gram positive and negative bacteria as test organism. The antioxidant activity can be done with bioassay technique by employing DPPH free radical and FRAP. *M. oleifera* extract was prepared with three different solvent distilled water, polar solvent (Methanol), Non-polar (hexane) and incubate for at least for 48 hours in incubator. Then the extract was filtered by using Whatmann paper and the crude extract is used for Phytochemicals screening, antioxidant assay and for synthesis of silver nanoparticles. The *M. oleifera* has a high content of phytonutrients therefore it is used to cure malnutrients as natural integrators. Phytochemicals such as Alkaloids, Saponins, Flavonoids, Terpenoids, Phenols are present in huge amount in *M. oleifera* leaf extract which have medicinal properties such as anti-carcinogenic, anti-diabetic, antioxidant, immunomodulatory, hepatoprotective and antiatherogenic. *M. oleifera* have been evaluated for its antioxidant activities. The antioxidant activity of the extract were evaluated by 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging activity assay. The synthesized silver nanoparticles also shown antioxidant activity. Their antioxidant activity was also evaluated by 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging activity assay. AgNPs are extensively used in many applications, mainly medical and biological applications. Synthesis of nanoparticles may serve as a future direction in biomedical nanotechnology in developing antimicrobial compounds, antioxidant compounds, treatment and prevention of diseases and significant advance in the diagnosis.

Keywords: *Moringa oleifera*, medicinal plants, green synthesis, nanoparticle, Antioxidant

1. Introductions
All the living form reliance on nature for their incumbent as being the inception for medicines, shelters, food, clothing, transport throughout their lives. For the huge proportions of world populations medicinal plants persist to show a paramount role in the healthcare structure and is mainly true in progressive countries, where herbal medicine has proceed with history of huge use. There are 35000-70000 plants species have at one time or another been used in some culture for medicinal purposes. Some medicinal plants holy basil, aloe vera, fenugreek, Ashwagandha, Amla, *M. oleifera*, turmeric, pepper, elachi, clove, ginger etc. Medicinal plants are priceless source of bioactive compounds and despite the contemporary progresses in pharmaceuticals and drugs development they remain a major proportion of our world. *M. oleifera*, drumsticks tree (From the long slender, triangular seed-pods), horseradish tree (from the taste of roots which resembles horseradish).

It has been studied for its health properties, attributed to the numerous bioactive components including vitamins, phenolic acids, flavonoids, isothiocyanates, tannis and saponins which are present in significant amount in various components of the plants. *M. oleifera* trees have been used to resist undernourishment, particularly among infants and nursing mothers. Scientific and the prominent literature have reports on the nutritional qualities of *M. oleifera* in huge number. *M. oleifera* has several medicinal properties and has potentiality to cure many disease. It is used to treat diseases such as diabetes, heart disease, anemia, arthritis, respiratory problems, skin, liver problems, paralysis, sterility, rheumatism, digestive disorders and many more. According to various research the leaves are said to be anti-fungal, anti-viral, anti-bacterial, anti-oxidative and acts as flocculating agents and stimulants *M. oleifera* powder can be used as substitute for iron tablet, hence a treatment for anemia. Silver nanoparticles are tiny particles of silver with a size range of 1 to 100 nanometers. They exhibit unique physical and chemical properties due to which their used in various fields are like medical, food, health care, consumer, and industrial purposes are rapidly increasing. Silver nanoparticles show antibacterial activity, catalytic activity, optical properties, electrical conductivity, magnetic properties, biocompatibility (Klaus Silver nanoparticles shows antibacterial activity against gram negative and gram positive bacteria. They also acts as catalysts in different chemicals because of their high surface area to volume and unique surface chemistry. They have unique optical properties like Plasmon resonance. They have high conductivity due to which they are used in electronic devices and conductive inks. They show low toxicity and biocompatibility in various vitro and in vivo studies. There are many different methods for synthesis of the nanoparticles but Green synthesis is eco-friendly and sustainable production of nanoparticles without using any hazardous chemicals.

2. Materials and Methods

2.1 Preparation of leaves extract

Healthy and fresh green leaves of *M. oleifera* was collected. The leaves are then washed three times with running tap water and dried at room temperature for 3-4 days to remove the moisture from the leaves and stored in sealed bag for further used. 10 grams of leaf extract was dissolve in 50 ml in polar, non-polar solvent, distilled water (non-polar solvent used n-hexane, polar solvent used methanol) and the mixture was incubate for at least for 48 hours (Hettich. *et al*, Germany). The mixture then filter using Whattman filter paper and the dry extract was collected and used to prepare nanoparticles.

2.2 Antioxidant analysis

The antioxidant activity of the *Moringa oleifera* extracts was evaluated by DPPH assay (2, 2-Diphenyl-1-picryhydrazyl). Firstly prepared DPPH solution by adding 0.3mM DPPH in 100% methanol. One milliliter of a 0.3 mM DPPH methanol solution was added to different concentration of 2.5 ml solution of the powdered leaf sample (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml) and allowed to react at room temperature for 30 min DPPH solution is used as control and methanol is used as blank. The absorbance was recorded at 517 nm by using Uv-Vis spectrophotometer. The antioxidant activity was expressed as the percentage of inhibition which was determined by using the following formula.

$$\%\text{of inhibition} = \frac{(\text{Control absorbance} - \text{Test absorbance})}{\text{Control absorbance}} \times 100$$

2.3 Phytochemical screening

Phytochemical analysis of Alkaloids, flavonoids, Phenols, Saponins, Tannins were performed by crude extract of *M. oleifera* leaf sample by adopting standard procedure depicted by Edeoga, and Trease and Evans.

2.4 Test for Alkaloids

To deduce the presence of alkaloids 2ml of leaf extract were added to 2 ml of conc. HCl and few drops of Mayer's reagent formation of green/white color precipitate confirms the presence of alkaloids.

2.5 Test for flavonoids

To deduce the presence of flavonoids, 2 mL of dilute 10% of Ammonia solution were added and then concentrated H_2SO_4. The detection of a yellow colouration in each sample indicates the presence of flavonoids.

2.6 Test for Saponins

To deduce the presence of saponin 2 ml of leaf extract were added to 2ml of distilled water. Then wait for 15 minutes after 15 minutes formation of 1cm thick foam confirms the presence of saponins.

2.7 Test for Tannins

To deduce the presence of Tannins 5ml of leaf extract was added to 2ml of chloroform then add 3ml of conc.H_2So_4 and wait for few seconds layer of reddish brown color indicate the presence of Tannins.

2.8 Test for Phenols

Alcoholic ferric chloride reagent was used for estimating the phenolic content of *M. oleifera* as depicted by (Siddhuraju and Becker) About 20 µg of the powdered leaf sample were taken 4-5 drops of alcoholic ferric chloride were added immediately bluish black is formed which confirm the presence of phenols (Siddhuraju P, Becker K 2003) [2].

2.9 Synthesis of silver nanoparticles

For synthesis of silver nanoparticles green synthesis method is followed. *M. oleifera* leaf extract is carried out by mixing the extract with a metal salt solution, we will use silver nitrate as metal salts. 1mM silver nitrate solution is prepared by dissolving silver nitrate in 100ml of distilled water. 2 mL *M. oleifera* leaves extract was added drop wise into 10 ml and 15ml of 1 mM aqueous solution of $AgNO_3$ under constant stirring. The formation of silver nanoparticles is indicated by the color change of the solution from yellow to reddish brown. The absorbance spectrum of the nanoparticles solution is recorded using a Uv-Vis spectrophotometer. The presence of peak in the spectrum at around 420nm confirms the formation of silver nanoparticles.

3. Results and Discussion

3.1 The result for qualitative analysis of *M. oleifera* leaf extract

Table 1.1: Showing result of phytochemical analysis of distilled water extract

Secondary Metabolites	Minimum	Moderate	Maximum	Color
Alkaloids			+++	Dark green
Flavonoids		++		Pale yellow
Phenol			+++	Black
Saponins		+		White foam
Terpenoids		++		Light brown

* All experiments performed in triplicates

Table 1.2: showing result of phytochemical analysis of methanol extract

Secondary metabolites	Minimum	Moderate	Maximum	Color
Alkaloids			+++	Dark green
Flavonoids			++	Greenish yellow
Phenol			+++	Black
Saponins			++	White foam
Terpenoids			+++	Dark brown

* All experiments performed in triplicates

Table 1.3: showing result of phytochemical analysis of n-hexane extract

Secondary metabolites	Minimum	Moderate	Maximum	Color
Alkaloids			+	Light green
Flavonoids			+	Light yellow
Phenol			+	Black
Saponins			+	White foam
Terpenoids			+	Light brown

All this experiments are performed in triplicates

The phytochemicals analysis carried out of *M. oleifera* leaves showed the presence of alkaloids, phenols, tannins, flavonoids, saponins which are the major secondary metabolites. This secondary metabolites are used in treatments of various diseases. Phenol is used as an oral analgesic, in cosmetics, in production of drugs, to ambalm bodies and are used as antiseptic for surgical instruments, also acts as antimicrobial agents. Tannin are used as astringent medicine for the treatment of intestinal disorder such as dysentery and diarrhea and it occurs in high percentage. Saponins medically used for treatment of the increased blood cholesterol and are beneficial to patient with arteriosclerosis and hypertension and in control of post-menopausal syndrome. Alkaloids can be used in the treatment of malaria, cough and cold, hypertension, diabetes and cancer, ant diarrheal, anticancer, anti-inflammatory and also helps in curing urinary disorder. The Phytochemical gives best result in methanol solvent as it has the capability of extracting lipophillic and hydrophilic molecule also have high extractability. The presence of some bioactive compounds are confirms similar result by I Ojeaga, HG Haruna *et al.* in 2014) [23] who in their study reported that *M. oleifera* contains tannins, phenols, alkaloids, flavonoids and saponins. Many studies are carried out to find out bioactive compounds of *M. oleifera* plant to investigate its medicinal properties which are useful for the living begins also their antioxidant, anti-inflammatory, anti-microbial, anti-cancer testing are also studied. (Moyo *et al* 2011, Wang *et al* 2000, Onwuliri 2004) [22, 21, 20]. *M. oleifera* have antifungal and antibacterial properties due to the presence of tannins therefore they are used in making antifungal and antibacterial drugs industry. It is also used in detergents industries as foaming agents due to presence of saponin in abundance amount also show high resistance against pest therefore used in pesticides (Cannon *et al*. 2004) [24].

3.2 Result for antioxidant analysis of *M. oleifera* leaf extract percentage of inhibition

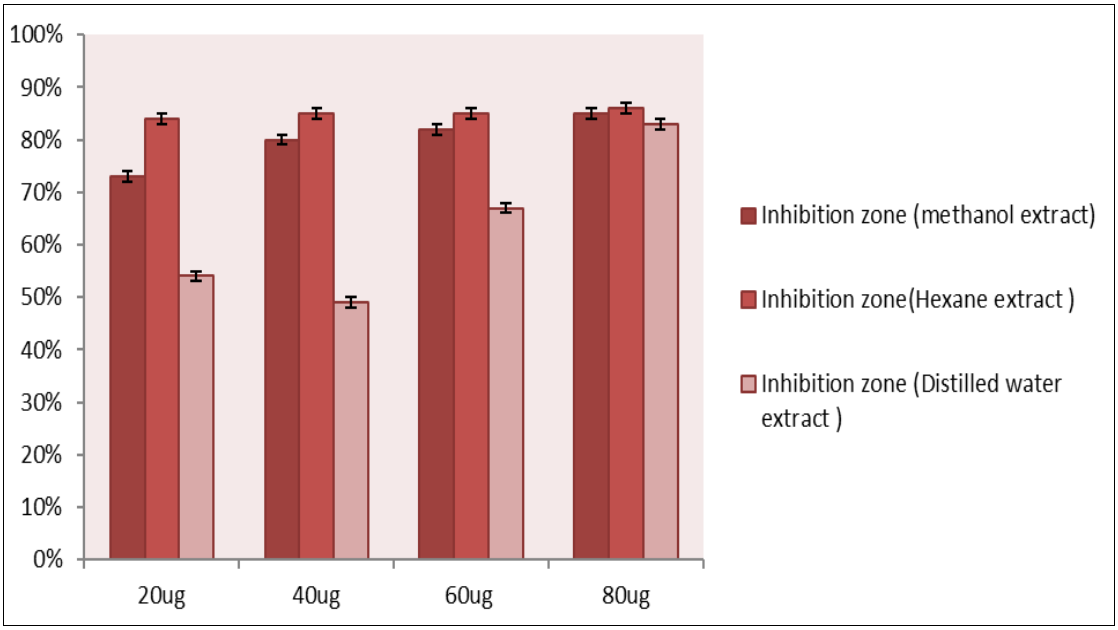
Table 1.4: Showing result of percentage inhibition

Concentration	Inhibition Percentage (methanol extract)	Inhibition Percentage (Hexane extract)	Inhibition Percentage (Distilled water extract)
20ug	73%	84%	54%
40ug	80%	85%	49%
60ug	82%	85%	67%
80ug	85%	86%	83%

*All this experiments are performed in triplets

The antioxidant analysis carried out of *M. oleifera* leaves possesses an interesting antioxidant activity and acts as a good source of antioxidant due to the presence of several type of compounds such as ascorbic acid, flavonoids, phenolic and cartenoids, alkaloids, tannins. Antioxidant activity plays a key role in the protection against cell oxidation. In this sense, particularly the antioxidants compounds founds in *M. oleifera* leaves can be used for the photo protection against oxidative stress caused by Uv exposure, which after prolonged exposure could lead to skin cancer. (Benzie IF, Strain JJ, 1999) [25]. The antioxidant properties of the plant is due to the presence of the phenolic compounds. Hydroxyl groups in ring B and 3-Oh group is related to flavonoids superoxide scavenging activity. (Siddhuraju P, 2003) [2] Studies antioxidant activity of *M. oleifera* from three different agro climatic condition got the same result of antioxidant. The 50% inhibitory concentration was expressed the quantity of the extract to react with half of the DPPH radicals. Linear regression were used to determined the IC50 value of *M. oleifera* extract. The IC50 value of methanol extract was 49.30ug/mL, n-Hexane was 715.21 ug/mL, distilled water extract 31.5 ug/mL. The n-Hexane show the maximum show the highest antioxidant activity as compared to methanol and distilled water extract this is due to the presence of phenolic compounds that contributed to antioxidant activity. The same study was conducted by (Wiwit Denny Fitriana *et al* 2016) [26] found the same result and found that *M. oleifera* can be used as antioxidant source. Xu *et al.* (2019) [27] conduct a comparative study on *M. oleifera* and *Olive olea* leaf and found the Moringa. oleifera show the highest antioxidant properties the *Olive olea* leaves and state that *M. oleifera* is the riches source of antioxidant and bioactive compounds that are useful for living begins also used as preservatives to extend the shelf-life of chicken burgers as they slowed down the deterioration of meat. Adefegha S, Oboh G, 2016 [28] conduct a study in which he collected *Moringa oleifera* leaves and analysis their antioxidant

property and found that different locations and environmental condition affect the plant antioxidant property. Aruoma OI (1999) [29] study the antioxidant properties at two different stage of plant growth at tender and mature stage and reported that mature leaves have high antioxidant property i.e. antioxidant properties vary with the stage of maturity.

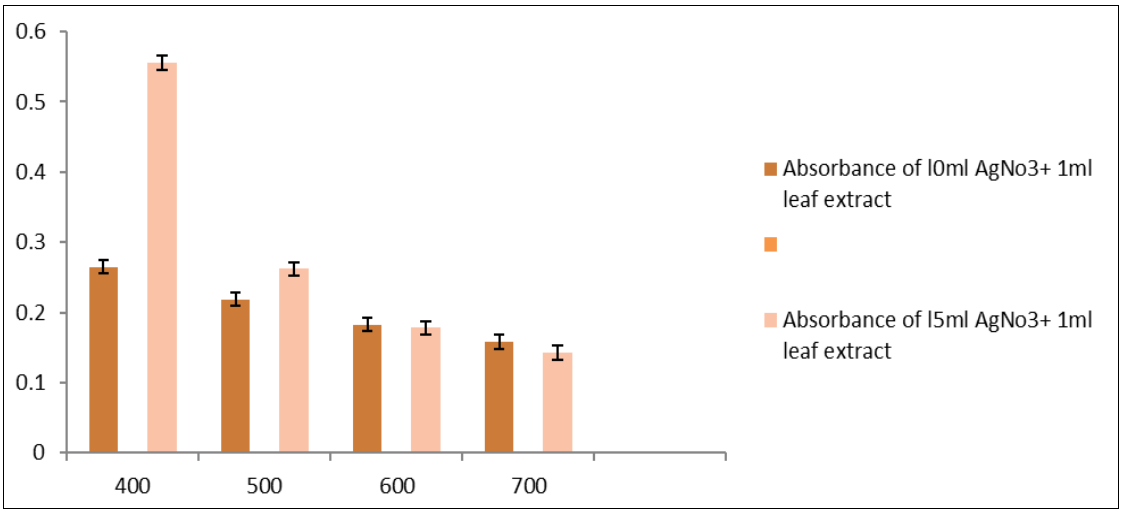


* All experiments performed in triplicates

Fig 1.1: Graph showing percentage of inhibition.

Table 1.5 showing result of spectrophotometer analysis of synthesis silver nanoparticles

	Absorbance at different concentration (Distilled water extract)	
Wavelengths	**10ml $AgNo_3$+ 1ml extract**	**15ml $AgNo_3$+ 1ml leaf extract**
400	2.353	1.902
500	1.864	1.076
600	1.402	0.805
700	1.237	0.641



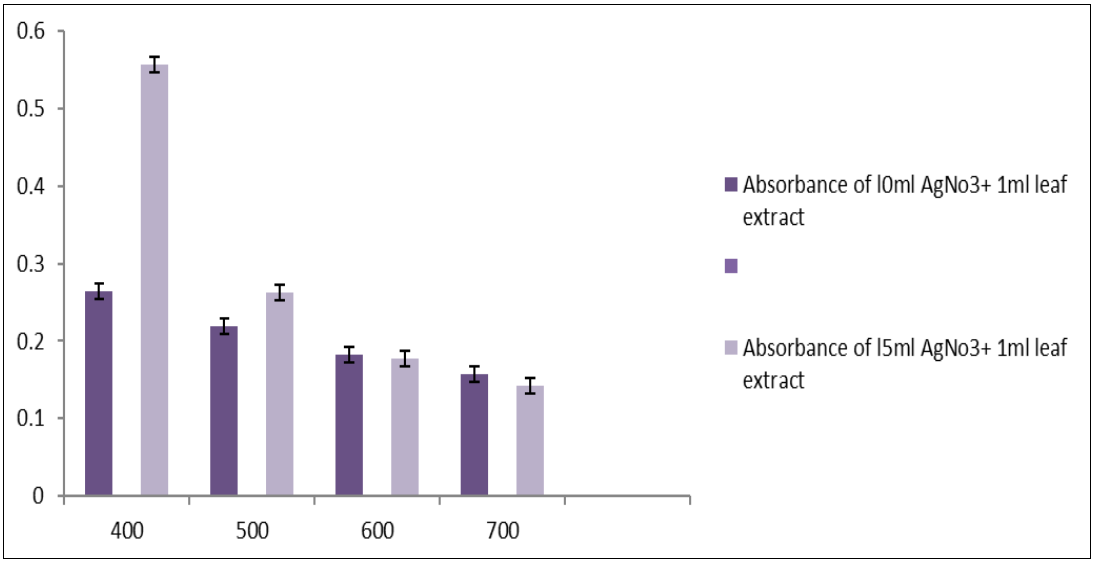
*All this experiments are performed in triplets

Fig 1.2: showing graph spectrophotometer characterization of silver nanoparticles

Table 1.6: showing result of spectrophotometer characterization of silver nanoparticles

	Absorbance at different concentration (Hexane extract)	
Wavelengths	**10ml $AgNo_3$+ 1ml extract**	**15ml $AgNo_3$+ 1ml leaf extract**
400	0.265	0.556
500	0.219	0.262
600	0.183	0.178
700	0.158	0.143

* All experiments performed in triplicates



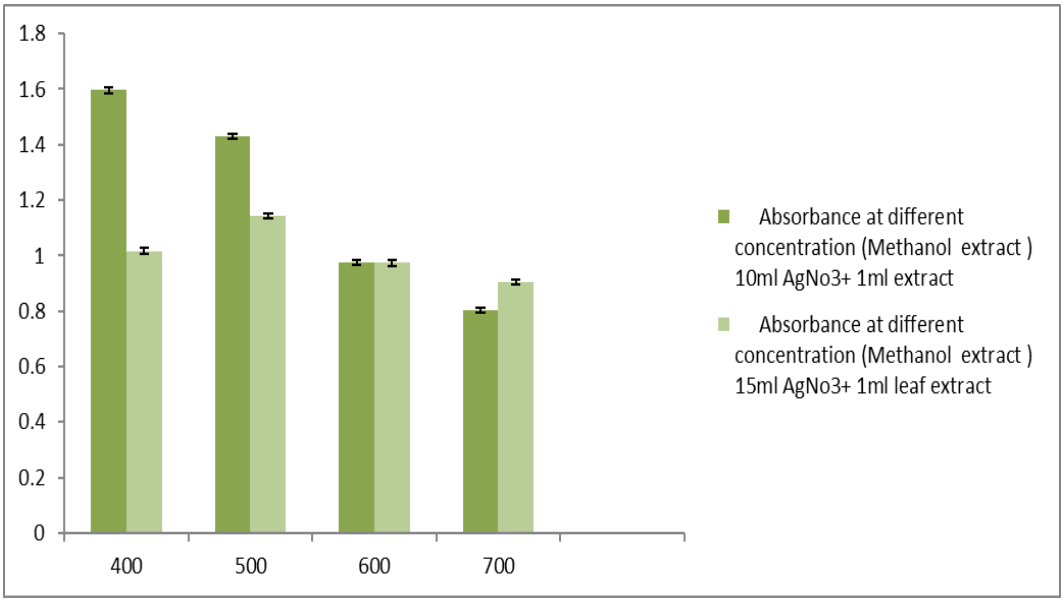
* All experiments performed in triplicates

Fig 1.3: showing graph spectrophotometer characterization of silver nanoparticles

Table 1.7: showing result of spectrophotometer characterization of silver nanoparticles

	Absorbance at different concentration (Methanol extract)	
Wavelengths	**10ml $AgNo_3$+ 1ml extract**	**15ml $AgNo_3$+ 1ml leaf extract**
400	1.596	1.016
500	1.429	1.142
600	0.976	0.973
700	0.804	0.905

All this experiments are performed in triplets



* All experiments performed in triplicates

Fig 1.4: showing graph spectrophotometer characterization of silver nanoparticles

The formation of silver nanoparticles confirms by the reddish brown color which is formed due to the chemical reaction Due to reduction of silver nitrate the formation of silver nanoparticles take place. Due to which the color of the solution containing silver nitrate turned yellowish to brown on heating, this color changes was due to the reduction of Ag^+ to Ag^0 by various biomolecules present in the leaf. The leaf extract contain Gallic acid this may be the reason why silver nitrate is reducing to silver nanoparticles and stability is achieved. Gallic acid is known to reduce metal salts to form nanoparticles. (Wang *et al*. 2007 [30], which confirms the formation of silver nanoparticles To confirm the formation of AgNPs, the reaction mixture was analysis in Uv-Vis spectrophotometer and the maximum absorption peak was observed at 400nm which confirms the formation of silver nanoparticles. Due to their unique physical and chemical properties there are extensively used in various area like in food industry, health care, medicinal and industry purposes. UV-Vis, FTIR, DLS, XRD and SEM analysis are most common methods of AgNPs characterization. (Pigłowski M 2019) [31]. The AgNPs has various biomedical application in cancer therapy, disease

control, infection control, dental application, wound healing and coating system with less or no side effects. They also show antioxidant, anti-microbial, anti-fungal, anti-bacterial properties. Bioactive compounds acts as stabilizing agent that prevents the electrostatic aggregation of the nanoparticles (Stohs SJ, Hartman MJ (2015) [18]. (Adhav, V.; Bhagare, 2022) [32] carried out a research in which they synthesized AgNPs from *Moringa oleifera* seeds. The color change of silver nitrate solution to reddish brown is due to surface Plasmon phenomena. Martinez-Castanon G, 2008 [33] conduct a research and reported that *Moringa oleifera* performs dual functionality acts as stabilizing and reducing agent. In transformative biomedical solutions *M. oleifera* plays a vital role. (Moodley JS 2018) [34] carried out a research in which *M. oleifera* and *Tamarindus indica* are used in formation of silver nanoparticles and analysis its anti-bacterial activity and report that they both show equally efficient on Staphylococcus aureus bacteria. Bindhu, MR (2020) [35] carried out a study in which *M. oleifera* flowers are used for synthesis of AgNPs and analysis its antimicrobial activity. The nanoparticles are used in various fields in biomedical, in treatment of cancer, in wound healing, in making scratch proof sunglasses, in detection of protein, in drug delivery, in wastage treatment, in tissue engineering. The green synthesis is cost effective, eco-friendly and sustainable method with minimal or no side effects.

4. Conclusion

M. oleifera is needed a very useful breakthrough in the demand of alternative natural medicine for the treatment of various disease activities by pathogenic organism. This is proved by the good antioxidant activity and the presence of secondary metabolites showed by the leaf extract. Therefore the plant could be used in the treatment of typhoid fever, diarrhea, stomach ulcer, tumors, post-menopausal syndrome, arteriosclerosis, control of blood sugar level etc. *M. oleifera* are the richest source of natural antioxidants. In reducing fat oxidation, inhibiting protein degradation and increasing fat stability and the shelf-life of chicken burgers, which are highly consumed products phenols plays important. Green synthesis which is regarded as a biological method of synthesizing nanoparticles was employed to silver nanoparticles from *M. oleifera* leaf extract which is a medicinal plant also known as drumstick. A number of biomolecules present in the leaf extract of *M. oleifera* are responsible for the stability of silver nanoparticles. Gallic acid is known to be found in leaves of the plant which reduced the silver present in the silver nitrate to form silver nanoparticles. The synthesized nanoparticles were analysis under Uv-Vis spectrophotometer under different wavelength and different concentration and every time the maximum peak was observed at 400nm. Which shown is due to a characteristic phenomenon known as surface plasma resonance which differs according to the concentration of silver nitrate and extract. It also confirm that the shape of synthesized nanoparticles was spherical.

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