WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 12, 1140-1166. Research Article ISSN 2277–7105

# PHYTOCHEMICAL SCREENING OF MORINGA OLEIFERA LEAF EXTRACT, FORMULATION, AND OPTIMIZATION OF SELENIUM NANOPARTICLES FOR ITS ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY

**Astha Dhawan***

Sagar Institute of Research & Technology – Pharmacy, SAGE University, Sahara Bypass Road, Katara Hills, Extension, Bhopal, Madhya Pradesh 462022.

Article Received on
04 May 2024,

Revised on 24 May 2024,
Accepted on 14 June 2024

DOI: 10.20959/wjpr202412-32844

***Corresponding Author**
**Astha Dhawan**
Sagar Institute of Research & Technology – Pharmacy, SAGE University, Sahara Bypass Road, Katara Hills, Extension, Bhopal, Madhya Pradesh 462022.

## ABSTRACT

Green nanoparticle production has several biomedical applications. This study aimed to extract powdered *Moringa oleifera* leaf material using distilled water at a 2:1 solvent-to-sample ratio. Selenium Nanoparticles (SeNPs) were synthesized by reducing Sodium Selenite with the aqueous leaf extract. This research work examined the plant extract's antioxidant, antibacterial properties, and phytochemical composition, along with the biosynthesis, characterization, and optimization of SeNPs. Analytical techniques such as DLS, SEM, UV-visible spectrometry, FT-IR analysis, TLC analysis, ANOVA, bacterial culture, and DPPH assay were employed to assess the physical attributes, antibacterial, and antioxidant properties of the synthesized SeNPs. Phytoconstituents like alkaloids, tannins, phenolics, sterols, and triterpenoids were identified in the extract, which had a total phenolic content of 65.39±0.78 GAE/mg. UV spectra showed absorption between 294-299 nm, while FTIR analysis indicated phenolic bands at 3272 cm-1. SeNPs size varied from 168.5-520.3 nm depending on extract concentration, with a polydispersity index of 0.261-0.614. SEM analysis revealed smooth, spherical particles. TLC analysis showed two bands with Rf values of 0.63 and 0.76. Optimal SeNPs were produced with 3% extract solution and 45 minutes of stirring. SeNPs exhibited the highest antibacterial activity with a 1.3 cm inhibition zone at 3% concentration. The DPPH test indicated mild to moderate antioxidant activity ranging from 6.34-21.26% RSA.

**KEYWORDS:** Green-Nanoparticle, *Moringa oleifera*, Selenium Nanoparticles (SeNPs), Sodium Selenite.

## 1. INTRODUCTION

Richard Feynman, a renowned physicist, in 1959 made a ground-breaking contribution to the field of nanotechnology as being the first physicist to lecture on "There's Plenty of Room at the Bottom." Since then, this lecture has become a seminal work in the field of nanotechnology and has inspired countless researchers to explore the possibilities of manipulating matter at the atomic and molecular levels. Feynman's visionary ideas have paved the way for significant advancements in the field of nanotechnology.[1] Nanotechnology is a fascinating field that explores the properties and behavior of materials at the nanoscale level. It encompasses a wide range of scientific domains, such as electronics, material science, physics, chemistry, biology, engineering, as well as information technology. Through investigation of the distinct characteristics that materials have at the nanoscale, researchers can develop novel technologies and applications that possess the capacity to completely revolutionize many industries.[2] At the nanoscale level, structures that are measured using nanotechnology typically range from 1 to 100 nm.[3] Nanoparticles have garnered significant attention due to their unique material properties resulting from their sub-microscopic size. Consequently, their practical utilization has extended to diverse domains like engineering, drug administration, nanomedicine, environmental safeguarding, and catalysis. Also, using nanoparticles to target the aforementioned conditions has demonstrated encouraging results in the treatment of melanoma, cardiovascular disease (CVD), skin illnesses, liver diseases, and many other conditions.[4]

The integration of nanotechnology in medicine has been found to significantly improve the efficacy and bioavailability of various drugs. This has led to the development of nanomedicines that are capable of delivering therapeutic agents to specific target sites in the body, resulting in enhanced therapeutic outcomes.[5] Since the late 1970s, the potential of nanoparticles in the field of biomedicine has been extensively studied and documented. This association has been coined as "nanomedicine" and has been the subject of over 10,000 publications. By 2005, there were nearly thirty papers available on this particular term.[6] In 2015, Web of Science reported a significant milestone in the field of nanomedicine, with over 1000 articles published after a decade or more of research. The majority of these articles

focused on the use of nanoparticles (NPs) for biomedical purposes, highlighting the growing interest in this area of study.[7]

In the field of nanotechnology, various advanced forms of nanoparticles are used as nanocarriers. Examples of these types of nanocarriers include liposomes, quantum dots, dendrimers, inorganic vectors, carbon nanotubes, hybrid nanoparticles, peptide-based nanoparticles, metal nanoparticles, polymer-based nanoparticles, and lipid-based nanoparticles. Due to their special qualities, these nanocarriers can be used in a variety of therapeutic, imaging, and drug-delivery applications.[8] The synthesis of nano-sized materials is a widely researched topic, with various methods available for their creation. Out of these approaches, two are commonly used: top-down and bottom-up synthesis. Using larger bulk materials and pulverizing them into nanoparticles is the top-down method. Conversely, the bottom-up method begins with individual atoms and works its way up to bigger nanomaterials. Both approaches have their advantages and disadvantages, and researchers are continually investigating and improving these techniques to develop fresh and innovative and because of their special qualities and possible uses, metal nanoparticles have attracted a lot of attention lately. Among the most commonly used metal nanomaterials are silver (Ag), gold (Au),[9] selenium (Se),[10] cadmium sulphide (CdS),[11] lead sulphide (PbS),[12] as well as iron oxide (Fe3O4).[13] These materials possess diverse advantageous characteristics that render them appropriate for a broad spectrum of uses.

For instance, silver and gold nanoparticles have excellent antibacterial properties, while selenium nanoparticles have been shown to possess antioxidant and anticancer properties. Cadmium sulphide and lead sulphide nanoparticles, on the other hand, have been used in solar cells due to their excellent light absorption properties. Iron oxide nanoparticles are useful in magnetic resonance imaging (MRI) due to their magnetic properties. Overall, metal nanomaterials have proven to be versatile materials with diverse applications in various fields.

In recent years, metallic nanoparticles have become a subject of great interest. Multiple studies have been carried out to examine the diverse advantages of these nanoparticles inside the realm of medicine. For instance, research has shown that metallic nanoparticles can be effective in fighting cancer (Anti-cancer agents),[14] as well as enhancing imaging contrast[15] and serving as drug carriers.[16] The augmentation of molar absorptivity in colloidal dispersions is a widely exploited characteristic of nanoparticles, with the intensity of their

surface plasmon resonance being a key factor in this phenomenon.[17] Notably, metallic nanoparticles like gold, silver, and copper are classic examples of this effect. The use of metallic nanoparticles as a means of drug delivery has gained significant attention in recent years. This is due to their unique properties such as high surface-volume ratio, stability, and functionality through chemical modifications of their surface. Additionally, metallic nanoparticles are relatively harmless, making them a promising option for drug administration. Because of their extensive history and simplicity in synthesis, metallic nanoparticles have attracted a lot of interest.

The process of nanoparticle coalescence, whereby particles with high surface energy merge to form larger bulk particles that are thermodynamically favoured, has been widely studied. The phenomenon of coagulation is observed between two metallic nanoparticles when repulsive forces are absent. The stabilisation of metallic nanoparticles is a crucial aspect in achieving spatial confinement of the particles within the nanoscale range. Steric exclusion and electrostatic stabilisation are two common methods for achieving this stabilisation. Electrostatic stabilisation can be aided by the addition of a capping agent, such as a ligand with the suitable functional groups, polymer, surfactant, or solid support. Similarly, steric exclusion can be achieved through the use of a capping agent. The assessment of nanoparticles is done by: Absorbance Spectroscopy, Infra-Red Spectroscopy, Transmission Electron Microscopy, Scanning Electron Microscopy, AFM, XRD, FT-IR, Extended X-ray Absorption Fine Structure, X-ray Photoelectron Spectroscopy.

In the fields of physics, chemistry, and biology, selenium (Se) is a crucial chemical element. Se is a naturally occurring element that comes in two different forms: organic (selenomethionine and selenocysteine) and inorganic (Selenite and selenium). Numerous studies have been conducted on these two forms of selenium because of their possible health advantages and toxicological impacts. While organic selenium is frequently found in plants and animals, inorganic selenium is usually found in soil and water. It is essential to comprehend how these two types of selenium differ from one another.

The element Selenium is known to exist in nature in two distinct polymorphic forms, namely crystalline and amorphous structures. The crystalline forms of selenium include monoclinic and trigonal structures. The present study concerns the characterization of monoclinic selenium (m-Se), which is known to exhibit a red coloration and is composed of rings of Se8. The subject under investigation exists in three distinct allotropic forms (α, β, and γ), each of

which is identified by its unique packaging. The study of trigonal selenium (t-Se), which is the most stable form of selenium in a crystalline form at ambient temperature. Notably, t-Se has a black colouring, which is intriguing because of its possible uses in a variety of industries. The chemical element selenium (Atomic number 34) exists in three non-crystalline forms: vitreous selenium, black amorphous, and red amorphous (a-Se). These forms lack a long-range ordered structure and exhibit amorphous characteristics. The red amorphous form is a reddish-brown powder that is produced by the rapid cooling of molten selenium. The black amorphous form is a black powder that is obtained by heating selenium.[18]

Glutathione peroxidase is an enzyme that functions as an antioxidant by protecting vital SH-groups and facilitating the breakdown of peroxides. Selenium is a key biochemical component of this enzyme. Selenium is thought to have bactericidal properties because it can catalyse the oxidation of intracellular thiols, which causes bacteria to perish. The mechanism of action involves the interaction of selenium with intracellular thiols, which are essential for the proper functioning of microbial cells. The oxidation of these thiols disrupts critical cellular processes, ultimately leading to cell death. This research has significant implications for the development of novel antimicrobial agents and highlights the potential of selenium as a therapeutic agent for the treatment of infectious diseases.[19-21]

The use of metal nanoparticles (NPs) in medicine has gained significant attention due to their potential therapeutic benefits. Among these, gold (Au) and silver (Ag) NPs have been extensively studied for their medicinal properties. However, the synthesis of these NPs can be costly, which limits their widespread use. On the other hand, selenium nanoparticles, also known as SeNPs, have gained attention as a potential alternative due to their cost-effectiveness and ability to enhance biological properties when combined with other agents. In a study conducted by Vahdati et al.20, it was demonstrated that the combination of selenium nanoparticles (SeNPs) and lysozymes exhibited a synergistic antimicrobial effect.[22] The higher surface-to-volume ratio of the particles makes their surface more exposed at the nanoscale, leading to a bigger rise in selenium activity in the nano-regime. In biological applications, SeNPs exhibit intriguing possibilities as antioxidants, cancer therapeutics, and drug carriers.[23] The degree of blue shift that nano-selenium can produce in the absorption spectrum varies depending on the preparation, as indicated by the selenium's absorption profile. As a result, it has been hypothesized that the bandgap of the element Se will increase from 1.7 eV in its bulk state to 3.3 eV when viewed at the nano-scale.[24]

Elemental selenium (Se) is the least poisonous form of selenium; hence its nano-form has garnered a lot of interest. It is interesting to note that functionalized SeNPs are less cytotoxic than inorganic selenium, selenate, selenite, seleno-proteins, and other forms of selenium.[25,26] Elemental nano-sized selenium may be preferable to conventional selenium sources in clinical trials.

The potential of SeNPs in the biomedical field has been widely recognized by researchers, who have emphasized the need for further investigation into their properties. SeNPs in particular have garnered attention for their extraordinary antibacterial activity; in fact, some research suggests that they might even be more potent than Ag NPs. As a result, there is a growing impetus to research SeNPs and their potential applications.[27-29] Several reviews have been published in the past few years on the biological applications of selenium nanoparticles and their compounds,[30] focusing especially on the techniques applied to their biological production.[31]

There have been several reported methods for creating selenium nanoparticles (SeNPs). Two major categories can be used to group reduction techniques used in different fields: chemical reduction and biological reduction. The employment of biological agents, such as bacterial and/or plant extracts, for the reduction of organic as well as inorganic selenium compounds, is one well-studied method in the field of biological reduction. The outcome of this process is the conversion of these compounds into non-toxic and beneficial selenium nanoparticles (SeNPs). The process of chemical reduction involves the utilisation of chemical reducing agents. The categorization of this method can be based on either the source of energy utilised or on the equipment utilised for reaction. These methods employed for the synthesis of the category in question have been primarily reported as hydrothermal, microwave, and sono-chemical.

A wild and cultivated variation of the genus Moringa that is a member of the family Moringaceae is called Moringa oleifera, Lam (synonym: M. pterygosperma Gaertn), which is commonly referred to as Moringa in literature. Moringa oleifera is a plant that has been used for centuries for its medicinal properties. It is one of the plant foods with the highest concentrations of vitamins A, B (1, 2, 3, 6, 7), C, D, E, and K. Moringa contains calcium, copper, iron, potassium, magnesium, manganese, and zinc, among other essential minerals. More than 40 natural antioxidants are present. Moringa's leaves, pods, seeds, gums, bark, and flowers are used to treat mineral and vitamin deficiencies, support a healthy cardiovascular

system, encourage normal blood sugar levels, neutralize free radicals (thereby lowering the risk of cancer), offer superior support for the body's anti-inflammatory mechanisms, enrich anaemic blood, and support the immune system. Moringa oil extracted from the seeds is also used in cosmetics due to its moisturizing and anti-aging effects on the skin. Furthermore, it enhances bone strength, mental clarity, and eyesight. It may help those with osteoporosis, menopause, depression, generalized weakness, malnutrition, and breastfeeding mothers. Additionally, it is used to create fertilizer, feed for cattle, and an effective fuel. Also, it is a very safe plant that is edible. The plant's ability to purify water has also made it an important tool for addressing water scarcity in developing countries. Overall, Moringa oleifera is a versatile plant with many potential benefits for human health and well-being.

## 2. MATERIALS AND METHODOLOGY

### 2.1. Collection of materials

The plant (Moringa oleifera) for the experiment was collected from the nearby areas of Bhopal. It was then authenticated by Saifia Science College, Bhopal.

### 2.2. Preparation of plant material

The plant leaves were washed and shade dried and were then powdered with simple grinding.

### 2.3. Extraction of plant material

**The extraction process was done via two procedures**

- Defatting Process by Soxhlet apparatus
- Maceration process of the defatted marc with distilled water

#### 2.3.1. Defatting process by soxhlet apparatus

Moringa oleifera powder (34.5g) was weighed and petroleum ether (350ml) was taken as an organic solvent for the extraction. For the defatting process, Soxhlet Apparatus was used and the extract was heated at $40^0$C. 25 siphon cycles were done for the collection of defatted marc was weighed 29.69g. It was then completely dried to remove any residual.

#### 2.3.2. Maceration process of the defatted marc with distilled water

The defatted marc which was obtained from the defatting process was taken and was put for maceration process with 300ml distilled water for a day. The obtained extract (250ml) was poured onto the muslin cloth into a 500 ml beaker and was put in the water bath for two days

for evaporation of excess liquid. After evaporation, the extract was collected in a watch glass having a syrup-like texture.

### 2.4. Phytochemical screening

The preliminary phytochemical screening analysis was carried out in the aqueous extract of *M. oleifera*. The reagents/tests used were: Alkaloid testing (Mayer's reagent, Dragandroff's test, Wagner's test), Tannins and Phenolics testing (Gelatin test, Ferric chloride test, Alkaline reagent test), Flavonoid testing (Shinoda test, Zinc hydrochloride reduction test, Alkaline reagent test), Proteins and Amino acid testing (Ninhydrin test), Sterols and Triterpenoid testing (Salkowski test), Glycoside testing (Froth test, Bontrager's test).

### 2.5. Total phenolic content

0.1g of Moringa oleifera extract was taken and mixed with 1.5 ml of methanol-water (50% methanol+50% water). Then the mixture was put for maceration for 2 hours with a foil-enclosed test tube and was shaken occasionally. After 2 hours, the mixture was filtered into a volumetric flask of 10ml. The volume of the filtrate was adjusted to 10 ml by adding a mixture of methanol and water. A precise volume of 0.2 ml of the solution was carefully combined with 1.8 ml of distilled water. Following this, a measured quantity of 0.3ml of Folin-Ciocalteu's reagent was introduced into the mixture. The mixture was then allowed to sit undisturbed for at least 5 minutes. After 5 minutes, 0.8ml of Sodium carbonate ($Na_2CO_3$) and 1.9ml of distilled water were added to the above solution and again put to rest for another 30 mins. After 30 min, the UV-visible spectrometer checked the solution's absorbance at 765 nm.[32]

Total phenolic content is calculated by the formula in Figure 2.1:

$$C=C_1 \times V/m$$

Where,

C= total phenolic content in mg/g, in GAE (gallic acid equivalent)

$C_1$= concentration of gallic acid established from the calibration curve in mg/ml

V= volume of extract in ml

m= the weight of the plant extract in g.

**[Figure 2.1: Formula for calculating Total Phenolic content].**

### 2.6. Formulation and Optimization of selenium nanoparticles

The Selenium nanoparticle formulation was optimized by using Design Expert 7.0.0 by applying a general factorial approach with two independent variables (Extract percentage at three levels and Stirring time at two levels). The design matrix was prepared as shown in Table 2.1.

**Table 2.1: Design matrix using general factorial approach.**

| S No. | Standard | Run | Block | Factor 1 A: Extract (%) | Factor 2 B: Stirring time (min) | Concentration of formulation code |
|---|---|---|---|---|---|---|
| 1. | 5 | 1 | Block 1 | 2 | 45 | F1 |
| 2. | 1 | 2 | Block 1 | 1 | 30 | F2 |
| 3. | 6 | 3 | Block 1 | 3 | 45 | F3 |
| 4. | 2 | 4 | Block 1 | 2 | 30 | F4 |
| 5. | 4 | 5 | Block 1 | 1 | 45 | F5 |
| 6. | 3 | 6 | Block 1 | 3 | 30 | F6 |

**The procedure for the formulation of selenium nanoparticles is as follows**

The M. Oleifera extract was taken and was divided into three solutions with 1%, 2%, and 3% concentrations, and a 25mM solution of Sodium Selenite ($Na_2SeO_3$) and 1 M Sodium Hydroxide (NaOH) was taken. 3ml of each extract concentration solution was taken and differentiated into 6 different beakers. To the above mixtures, 3 ml of sodium selenite was added with 0.1g of polyvinyl alcohol (PVA) and was diluted with 9 ml of distilled water into each of the above differentiated 6 beakers with different extract concentrations. 1M NaOH was added to all the above beakers drop by drop until the solution became alkaline (colour change indicates pH change). The above 6 differentiated beakers of different solution concentrations were then stirred on a mechanical stirrer for 30 mins and 45 mins making it two 1% extract solution beakers will be stirred at 30 min and 45 min and the same was followed for all the other extract solutions.[33]

### 2.7. Characterization of the selenium nanoparticles

**2.7.1 UV-visible Spectroscopy:** A UV spectrophotometer was used to estimate the absorbance of each nanoparticle sample. The absorption spectra measured between 200 and 1100 nm in wavelengths. The blanking process was carried out using distilled water. Each sample was used separately and inserted into the spectrophotometer at a volume of 1 ml. A Double beam spectrophotometer was used for the characterization.

The peaks of SeNPs that were created from the leaf extract of Moringa oleifera were evaluated.

**2.7.2 Particle size Analyzer (dynamic light scattering):** Dynamic light scattering (DLS) is often referred to as PCS or photon correlation spectroscopy. DLS was utilized to calculate the hydrodynamic diameter of SeNPs by calculating the Brownian motion time scale as a result, the average size of the NPs was determined using the polydispersity index (PDI) by using Malvern Particle Size Analyzer MS2000.[34]

**2.7.3 Scanning electron microscopy:** A high-energy electron beam is scanned in a raster scan pattern using a scanning electron microscope (SEM) to create an image on the material. To determine the nanoparticles' size, shape, and surface morphology, scanning electron microscopy was used. Pictures were captured using a SEM at 15 kV and 8000x magnification.[35,36]

**2.7.4 FT-IR Analysis:** To detect organic, polymeric, and rarely inorganic materials, an analytical technique called Fourier Transform Infrared Spectroscopy (FTIR Spectroscopy) is employed. The Bruker ALPHA FTIR Spectrometer was utilized to ascertain the sample's molecular fingerprint. Following this, the sample specimens underwent examination through Fourier transform infrared spectroscopy (FT-IR) in a suspension form (0.1 ml, 2 drops) within a range of wavelengths from 400 to 4000 cm−1, via a resolution of 4 cm-1. The resulting signal at the detector appears as a spectrum, which is the sample's molecular fingerprint.[37]

**2.7.5 TLC Analysis:** Combinations that are not volatile are separated using the thin-layer chromatography method. Additionally, it is the fastest, most cost-effective, and simplest approach. The plate undergoes a stationary phase designed to desiccate and stabilize it. The chromatographic purification TLC technique was employed to separate and identify the main constituents contained in the most potent plant extracts. As the solvent system, a mixture of Chloroform, methanol, and ethanol with a ratio of 2:2:0.5 was used.[38] For the application of sample spots, thin pencil marks were formed at the plate's base. The specified regions were subjected to the sample solutions. The mobile phase was transferred into the TLC chamber and a moistened filter paper was inserted to ensure uniform humidity. The plate was placed into the TLC chamber, with the lid shut, and maintained so that the sample faces the mobile

phase. After observing the appearance of spots, plates were removed and allowed to dry.[39]

**2.7.6 Anova analysis:** Systematic variables and random factors are the two groups into which the observed aggregate variability found in a data collection is separated by the statistical analysis tool known as analysis of variance (ANOVA). The Formula for ANOVA is:

$$F = MSE/MST$$

Where,

F=ANOVA coefficient

MST=Mean sum of squares due to treatment

MSE=Mean sum of squares due to error

**[Figure 2.2: Formula for ANOVA]**

Doing an ANOVA test is the initial step in evaluating factors in a data set. The purpose of the test is to compare more than two groups and determine whether there is a correlation between them. The analysis of variability within and between samples is made possible by the F statistic, sometimes referred to as the F-ratio. The null hypothesis proposes that there is no significant disparity between the groups being examined. The ANOVA is indicated by an F-ratio statistic that is close to 1. With the numerator and denominator degrees of freedom serving as its two characteristic parameters, the distribution of all possible F statistic values is known as the F-distribution.[40]

## 2.8. Antibacterial screening of the selenium nanoparticles

**2.8.1 Preparation and Sterilization of nutrient medium:** 76g of agar agar was weighed and 120 ml of distilled water was added to it and then mixed thoroughly till there is no residue left. 6 plates were taken for the bacterial culture. These plates were and wrapped using aluminum foil then tied so there is no space from where the water can seep inside the plates. The plates and the agar agar mixture were then put into the autoclave for sterilization at 121$^{o}$C. After the sterilization was completed, the plates were taken out and were then put in the laminar airflow.

**2.8.2 Inoculation of bacterial culture to Plate and Application of sample:** The laminar airflow was sanitized with 70% ethanol and 30% water solution. UV light was used for 15-20 minutes for sterilization. Plates and agar agar mixture were then placed inside. The agar agar mixture was poured into the plates, and small cups were made at the center. A soil sample with bacteria was spread around the cups on each plate. Selenium nanoparticles were administered into the cups. The plates were then placed in incubation for 48 hours to observe bacterial growth and test for anti-bacterial activity shown by the nanoparticles.

### 2.9. DPPH Scavenging testing for Anti-oxidant activity

The antioxidant function of SeNPs was examined using the DPPH free radical scavenging assay. 1 mM DPPH solution in ethanol and a test solution of 10 ml (0.1 ml of extract of 1% + 9.9 ml of water) for each of the samples was prepared. 1.5 ml of DPPH solution was taken with 15 ml of test solution. It was then incubated for 30 mins at 37ºC and after 30 mins the absorbance of all samples was measured at 517 nm. A blank was run with 3 ml of ethanol solution and as a control, 3 ml of DPPH solution was made earlier without the sample being used. Then DPPH activity i.e., free radical scavenging activity was calculated by photometric analysis.[41]

$$\text{DPPH Activity (\%)} = \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \times 100$$

Where,

Abs control = Absorbance of control reaction.

Abs test = Absorbance in the presence of the samples of extract.

**[Figure 2.3: Formula for calculating free radical scavenging activity]**

## 3. RESULT AND DISCUSSION

### 3.1. Results of phytochemical testing

From the below phytochemical testing results, it was concluded that the Moringa oleifera extract had Alkaloids, Tannins, Phenolics, Sterols, and Triterpenoids, similar to the previously reported data.

| S No. | Phytochemical tests | Observation | Inference |
|---|---|---|---|
| **Alkaloid Testing** | | | |
| 1. | Mayer’s reagent | No Cream precipitate | Negative |

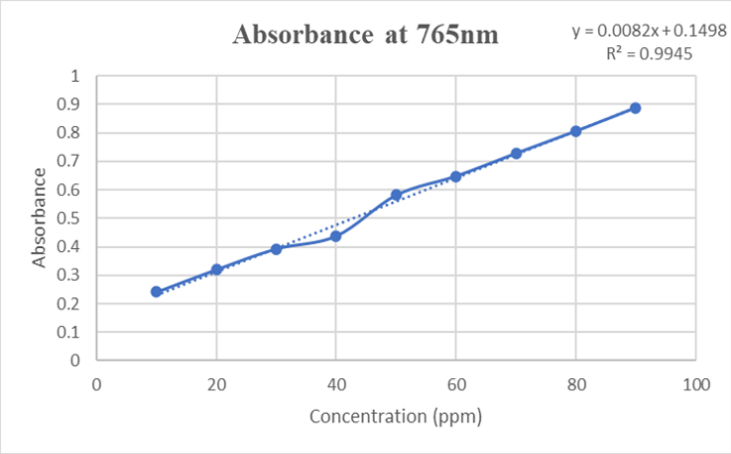
| | | | |
|---|---|---|---|
| 2. | Dragandroff's test | No Reddish-brown precipitate | Negative |
| 3. | Wagner's test | Reddish brown precipitate was obtained | Positive |
| **Tannins and phenolic testing** | | | |
| 4. | Gelatin test | No White precipitate | Negative |
| 5. | Vanillin test | No Blue green colour | Negative |
| 6. | Alkaline reagent | Yellow to red precipitate was obtained | Positive |
| **Flavonoids testing** | | | |
| 7. | Shinoda test | No Pink scarlet, crimson red or occasionally green to blue colour | Negative |
| 8. | Zinc HCl reduction | No Red colour | Negative |
| 9. | Alkaline reagent | No Intense yellow turns colourless on addition of dil. acid | Negative |
| **Protein and Amino acid testing** | | | |
| 10. | Ninhydrin test | No Violet colour | Negative |
| **Sterols and triterpenoid testing** | | | |
| 11. | Salkowski test | Red colour in lower layer=steroid<br>Yellow colour in lower layer=triterpenoid was obtained | Positive |
| **Glycoside testing** | | | |
| 12. | Froth test | No Frothing is seen (Saponin glycoside) | Negative |
| 13. | Bontrager's Test | No Rose pink or red colour in the ammonical layer (anthraquinone glycoside) | Negative |

### 3.2. Results of TPC (Total Phenolic content) testing

Data on absorption were obtained by calculating and plotting the gallic acid standard curve in distilled water (Table: 3.1). Regression coefficient and Beer's law range were ascertained from this. It was discovered that the linear equation for Gallic acid was y = (0.0082x + 0.1498) and the Regression Coefficient was found to be $R^2$ = 0.9945 [Figure: 3.1]. The TPC in the extract was calculated to be 65.39333± 0.782607 GAE/mg. Previous studies on the dried leaf extract have also revealed that Total Phenolic Content in the aqueous form of the dried leaf extract was found to be equivalent to our results.

**Table 3.1: Absorbance data of gallic acid (at 765 nm).**

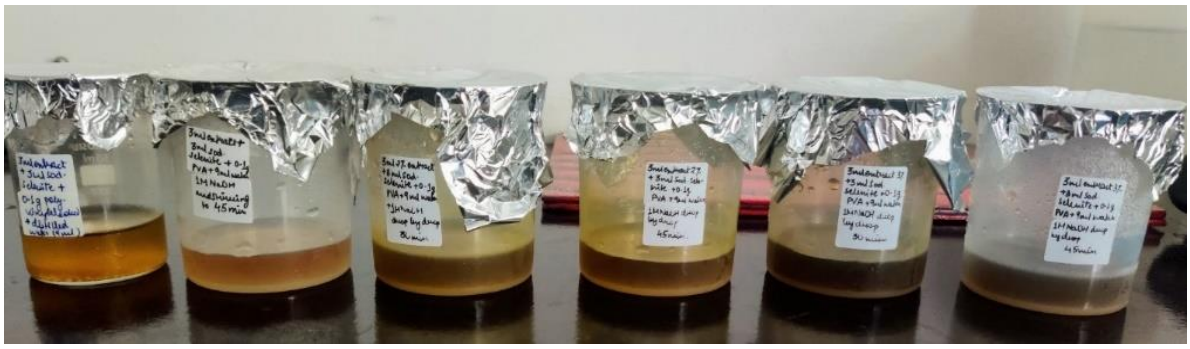
| Concentration (ppm) | Absorbance at 765nm |
|---|---|
| 10 | 0.241 |
| 20 | 0.32 |
| 30 | 0.393 |
| 40 | 0.438 |
| 50 | 0.581 |
| 60 | 0.648 |
| 70 | 0.728 |
| 80 | 0.806 |
| 90 | 0.888 |




**[Figure 3.1: Calibration curve of gallic acid]**

### 3.3. Bio-synthesis of SeNPs (Selenium nanoparticles)

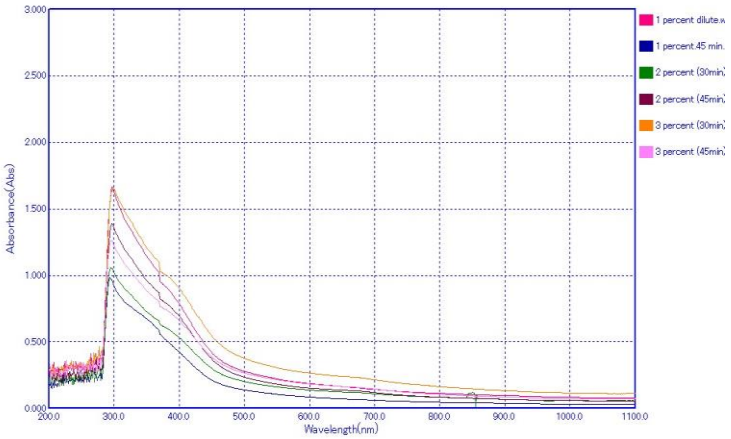
Se-NPs were produced quickly and with a brown to yellow colour shift when sodium selenite was reduced in the presence of Moringa oleifera extract during the biosynthesis process. Since polyvinyl alcohol (PVA) molecules help control particle size and stop nanoparticle aggregation, they were employed as the stabilising agent. Using varying volumes of Moringa oleifera leaf extract as the reducing agent resulted in a change in color for the Se NP solutions. This illustrates how reducing agents function in the creation of selenium nanoparticles. The extracted results were seen to be solutions with a brownish-yellow hue [Figure 3.2].



**[Figure 3.2: SeNPs synthesized using different concentrations of *Moringa oleifera* extracts].**

### 3.4. Characterization of SeNPs

**3.4.1. UV-absorption:** The UV spectrum of various SeNPs synthesized using the different concentrations of extract of Moringa oleifera was revealed at the absorption maxima at 294-299 nm suggesting the completed synthesis of SeNPs [Figure 3.3].

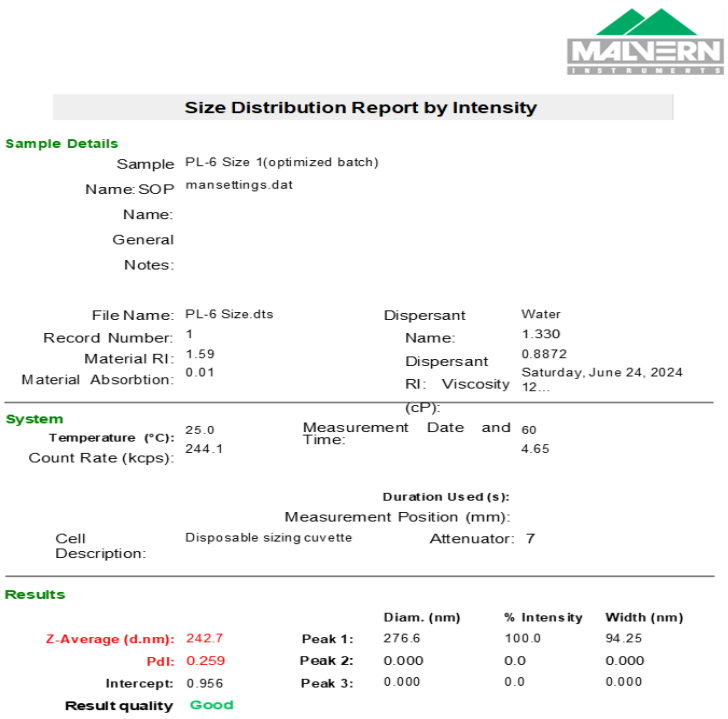



**[Figure 3.3: UV spectrum of SeNPs synthesized at different concentrations using *Moringa oleifera* dried leaf extract as reducing agent].**

**3.4.2. Particle Size and Distribution:** The particle size was identified through the utilisation of a Malvern particle size analyzer. The particle size of the SeNPs nanoparticles was found to decrease with an increase in the concentration of the reducing agent (extract) as indicated by a more brownish color of the solution and an increase in stirring time was found to decrease the particle size except when 3 % extract was used (particle size increased with stirring time). The particle size ranged from 168.5 to 520.3 nm in various samples with varying polydispersity index (PDI) [Table 3.2]. When Moringa oleifera dried leaf extract was utilised to synthesise the SeNPs, smaller particles were produced. The extract concentration had no effect on the PDI. Figure 3.4 shows a typical particle size by intensity graph obtained for SeNP F1 and F6 using the Particle Size Analyser.

**Table 3.2: Selenium nanoparticles using moringa oleifera extract.**

| Formulation | Particle size (nm) | PDI |
|---|---|---|
| F1 | 520.3 | 0.614 |
| F2 | 465.3 | 0.513 |
| F3 | 369.8 | 0.261 |
| F4 | 321.4 | 0.286 |
| F5 | 168.5 | 0.422 |
| F6 | 242.7 | 0.259 |



Size Distribution Report by Intensity

Sample Details

| | | | |
|---|---|---|---|
| Sample Name: | PL-6 Size 1(optimized batch) | | |
| SOP Name: | mansettings.dat | | |
| General Notes: | | | |
| File Name: | PL-6 Size.dts | Dispersant Name: | Water |
| Record Number: | 1 | | 1.330 |
| Material RI: | 1.59 | Dispersant RI: | 0.8872 |
| Material Absorbtion: | 0.01 | Viscosity (cP): | Saturday, June 24, 2024 12... |

System

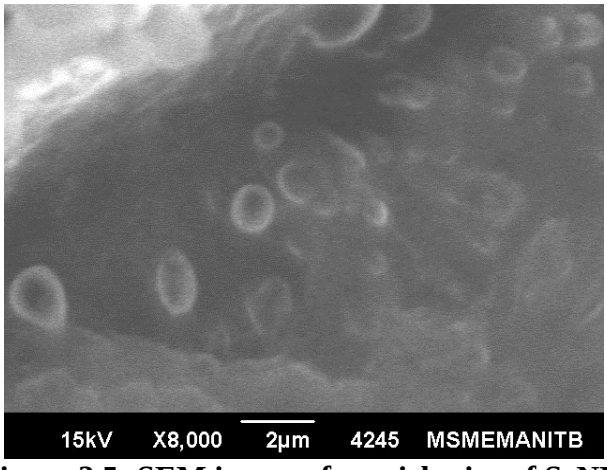
| | | | |
|---|---|---|---|
| Temperature (°C): | 25.0 | Measurement Date and Time: | 60 |
| Count Rate (kcps): | 244.1 | | 4.65 |
| | | Duration Used (s): | |
| | | Measurement Position (mm): | |
| Cell Description: | Disposable sizing cuvette | Attenuator: | 7 |

Results

| | | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 242.7 | Peak 1: | 276.6 | 100.0 | 94.25 |
| PdI: | 0.259 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: | 0.956 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality | Good | | | | |

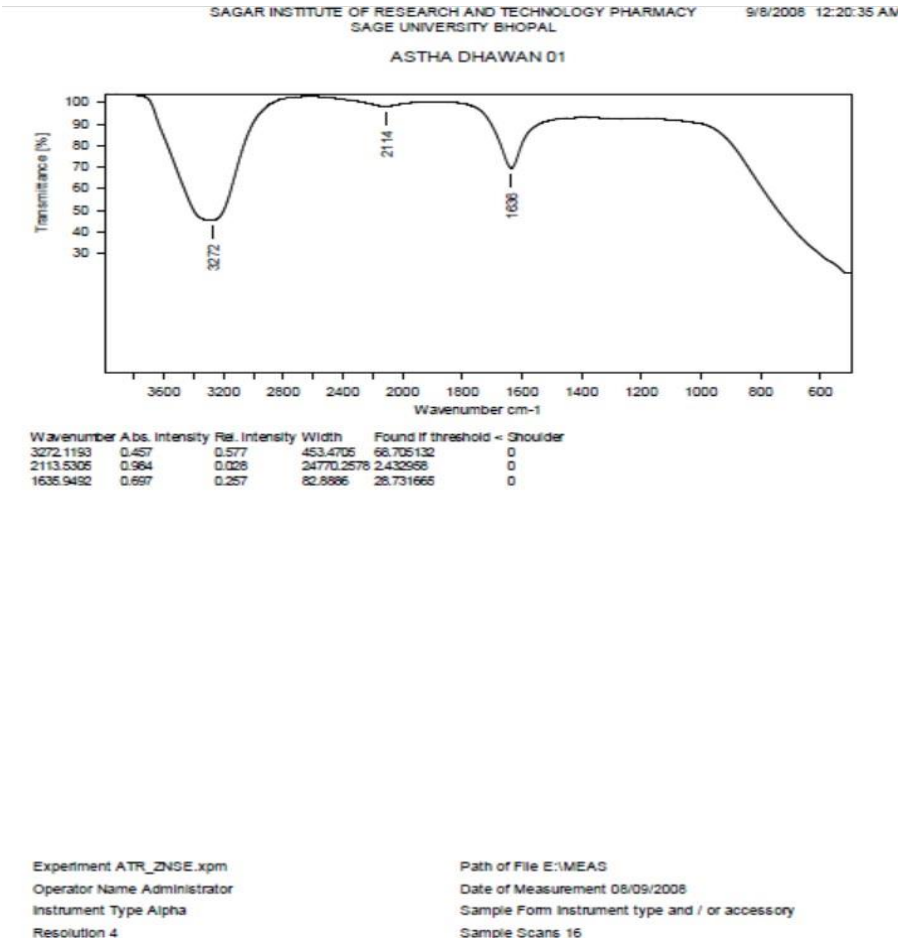
**Figure 3.4: Size distribution report of optimized batch F6.**

**3.4.3. Surface Morphology of Se-NPs:** With the use of SEM imaging, the surface of the SeNPs was investigated. Smooth particles with a spherical shape were visible in the SEM image. Scanning electron microscopy investigation revealed that the produced nanoparticles were spherical in shape and that the Se-NPs were uniformly dispersed. Images were captured with an 8,000X magnification at 15 kV. Based on SEM examination, the size of M. Oleifera SeNPs was determined to be between 2 and 5µm.The spherical morphology is vital for cellular uptake of the particles and is helpful in maintaining minimum energy thereby resulting in the stable particles [Figure 3.5].




**[Figure 3.5: SEM image of particle size of SeNP].**

**3.4.4. FT-IR Analysis:** The FT-IR analysis was done on the optimized nanoparticle size obtained from the 6 different extract concentrations. The resulting signal at the detector presents as a spectrum, typically from 4000 cm-1 to 400 cm-1, representing a molecular fingerprint of the sample. The FT-IR analysis of SeNPs indicates the absorbance bands from 3272.11–1635.94 $cm^{-1}$ with the phenolic band at 3272 $cm^{-1}$ (Range: 3100 cm-1 to 3500 cm-1) shown in Figure: 3.6.




**[Figure 3.6: FT-IR analysis report by Bruker ALPHA FTIR Spectrometer]**

**3.4.5. TLC Analysis:** The extracts were dissolved in water to achieve a concentration of 3 ml. The aqueous extracts of *M. oleifera* were applied using a Hamilton syringe on aluminium sheets pre-coated with silica gel 60 F254 of 0.2 mm thick, 4 cm x 10 cm used as a stationary phase. The plates developed in the chloroform, methanol, and ethanol solvent system in the ratios 2:2:0.5 showed 2 bands at 0.63, and 0.76 of Rf values respectively with the aqueous extract indicating the presence of phenolic compound shown in Figure: 3.7.

**[Figure: 3.7 TLC analysis for the presence of Phenolic compound in the plant extract]**

**3.4.6. Anova analysis:** The equation obtained from the ANOVA for the optimum particle size was found to be:

**Particle size=144.80×A -4.87×B+ 348**

Where,

A=Extract concentration

B=Stirring time

Intercept= 348

Hence, according to this formula, the optimized batch of SeNPs was found to be of 3% extract solution with 45 min of stirring time having maximum desirability. The report below represents the ANOVA analysis.

**Table 3.3: Analysis of variance table [Classical sum of squares - Type II].**

| Source | Sum of squares | Mean (df) | Mean Square | F-value | p-value | Prob > F |
|---|---|---|---|---|---|---|
| Model | 82643.23 | | | 10.41 | 0.0889 | not significant |
| A-Extract concentration | 82501.12 | 3 | 27547.74 | 15.58 | 0.0603 | |
| B-Stirring Time | 142.11 | 2 | 41250.56 | 0.054 | 0.8383 | |
| Residual | 5294.49 | 1 | 142.11 | | | |
| Cor Total | 87937.72 | 2 | 2647.25 | | | |
| | | 5 | | | | |

The 8.89% probability that noise might produce a "Model F-value" this high is indicated by the Model F-value of 10.41. Significant model terms are indicated by "Prob > F" values less than 0.0500. There are no significant model terms in this case. Values surpassing 0.1000 indicate a lack of significance between the model terms. Model reduction can significantly

enhance the performance of the model by eliminating unnecessary terms that do not contribute to its overall hierarchy.

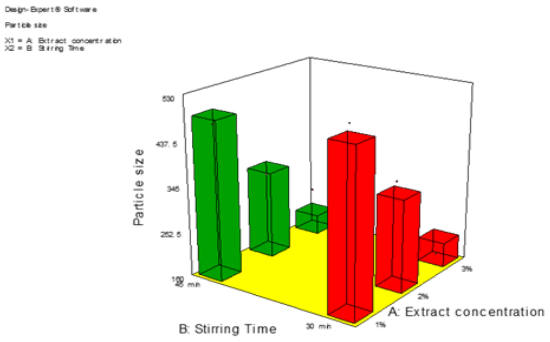
| | | | |
|---|---|---|---|
| Std. Deviation | 51.45 | R-Squared | 0.9398 |
| Mean | 348 | Adj R-Squared | 0.8495 |
| C.V. % | 14.78 | Pred R-Squared | 0.4581 |
| PRESS | 47650.44 | Adeq Precision | 7.068 |

**(a)** **(b)**

**[Figure: 3.8 (a) and (b) Factors effecting ANOVA analysis].**

**Final equation in terms of coded factors**

**Particle Size= 144.80×A -4.87×B+ 348**




**[Figure 3.9: 3D-Surface plot of influence of variables on particle size]**

**Table 3.4: 6 predicted solutions for minimizing particle size of the formulation.**

| S. No | Constraints name | Goal | Lower limit | Upper limit |
|---|---|---|---|---|
| 1. | Extract Concentration | Is in range | 1% | 3% |
| 2. | Stirring time | Is in range | 30 min | 45 min |
| 3. | Particle Size | minimize | 168.5 | 520.3 |

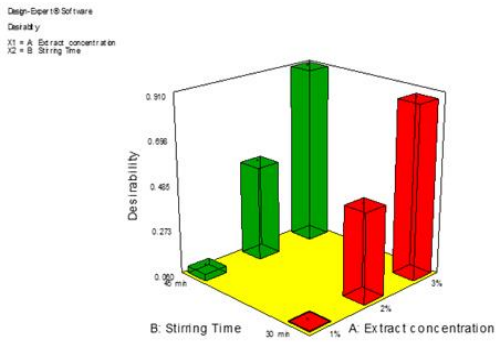
**Table 3.5: Solutions for 6 combinations of categoric factor levels.**

| Number | Extract concentration | Stirring time | Particle size | Desirability |
|---|---|---|---|---|
| 1. | 3% | 45 min | 200.733 | 0.098 (selected) |
| 2. | 3% | 30 min | 210.467 | 0.881 |
| 3. | 2% | 45 min | 340.733 | 0.51 |
| 4. | 2% | 30 min | 350.467 | 0.483 |
| 5. | 1% | 45 min | 487.933 | 0.092 |
| 6. | 1% | 30 min | 497.667 | 0.064 |

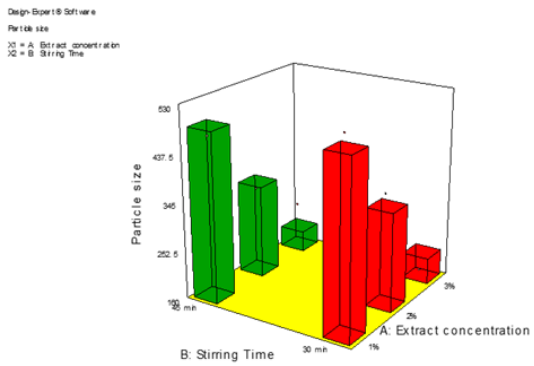
**6 Solutions found**

**Table 3.6: Number of starting points: 6 for 6 combinations of categoric factor levels.**

| Extract concentration | Stirring |
|---|---|
| 1% | 30 min |
| 2% | 30 min |
| 3% | 30 min |
| 1% | 45 min |
| 2% | 45 min |
| 3% | 45 min |

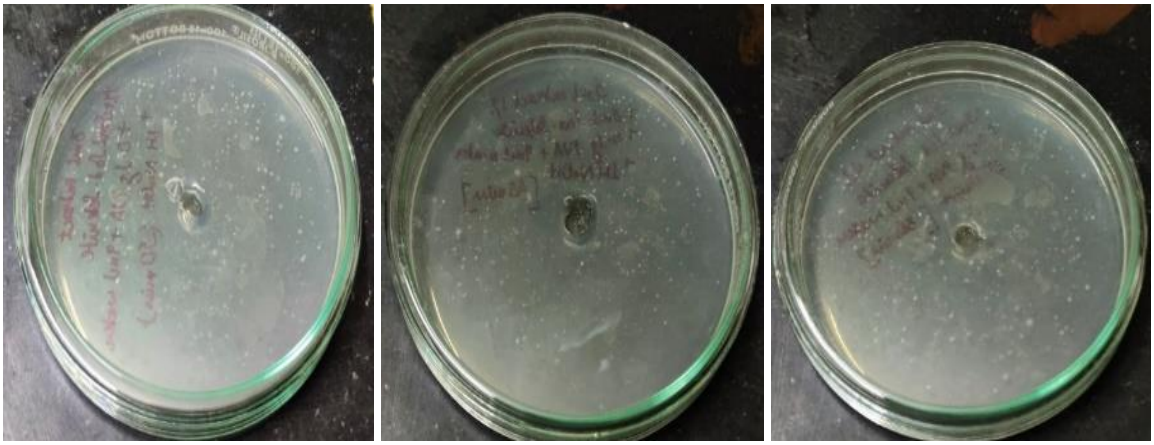



**[Figure 3.10: 3D- a surface plot of Desirability plot of solution].**




**[Figure 3.11: 3D- surface plot of particle size with predicted solution]**

**3.4.7. Anti-Bacterial activity:** The anti-bacterial activity was tested against six bacterial cultures. Bacterial cultures were created using soil samples from the home garden. Results showed that zone of inhibition (ZOI) in NPs of Selenium inhibited microbial growth. Significant ZOI was revealed by SeNPs, and the extent of the zone grew larger as SeNP concentration increased [Figure 3.12]. SeNPs produced at a concentration of F5 demonstrated more substantial results [Table 3.7].



(a) (b) (c)

(d) (e) (f)

**[Figure 3.12: a) 1% extract (30 min); b) 1% extract (45 min); c) 2% extract (30 min); d) 2% extract (45 min); e) 3% extract (30 min); f) 3% extract (45 min)]**

**Table 3.7: Zone of inhibition by the different percentage of extract solutions of Moringa oleifera dried leaf.**

| S No. | Extract % | Stirring time | Zone of inhibition (cm) |
|---|---|---|---|
| 1. | F1 | 30 min | 0.2 |
| 2. | F2 | 45 min | 0.3 |
| 3. | F3 | 30 min | 0.5 |
| 4. | F4 | 45 min | 1 |
| 5. | F5 | 30min | 1.3 |
| 6. | F6 | 45 min | 1 |

**3.4.8. Anti-oxidant activity (DPPH assay):** Using the DPPH test at 517 nm, the free radical scavenging activity was evaluated. The control was taken to be a DPPH solution in ethanol (Absorbance at 0.489) without any SeNPs. Results are displayed in Table 3.8. The outcomes showed that selenium NPs' antioxidant activity is shown to

be mild to moderate as shown in Figure 3.13. Suggesting that the extract was incorporated into the nanoparticles during synthesis.

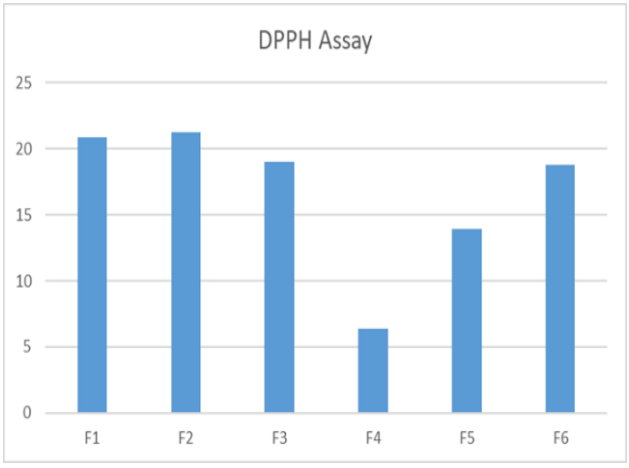
**Table 3.8: (a) and (b) Results of DPPH assay suggesting the antioxidant activity.**

**(a)**

| S No. | Sample name | Wavelength (nm) | Absorbance |
|---|---|---|---|
| 1. | F1 | 517 | 0.591 |
| 2. | F2 | 517 | 0.593 |
| 3. | F3 | 517 | 0.582 |
| 4. | F4 | 517 | 0.520 |
| 5. | F5 | 517 | 0.557 |
| 6. | F6 | 517 | 0.581 |

**(b)**

| S No. | Sample name | DPPH assay (%) |
|---|---|---|
| 1. | F1 | 20.85 |
| 2. | F2 | 21.26 |
| 3. | F3 | 19.01 |
| 4. | F4 | 6.34 |
| 5. | F5 | 13.90 |
| 6. | F6 | 18.81 |




**[Figure 3.13: Representation of Antioxidant activity shown by various extract % of *Moringa oleifera* dried leaf].**

## 4. CONCLUSION

Currently, nanotechnology is gaining significant attention as a rapidly developing field due to its potential applications in various aspects of life. The literature provides evidence of the numerous beneficial applications of NPs. Nanoparticles (NPs) are fabricated utilizing a variety of materials, including polymers, ceramics, and lipids, among others. Nanoparticles of

varying sizes exhibit antimicrobial, antioxidant, and anticancer properties, among other potential applications. A range of methodologies are utilized in the production of nanoparticles, encompassing physical, chemical, and biological approaches.[35]

However, numerous concerns arise with respect to the toxicity and lack of environmental sustainability associated with physical and chemical techniques. A safer and non-toxic method is necessary. The biological approach to synthesizing nanoparticles is the preferred method. Compared to alternative methods, it possesses numerous advantageous and beneficial aspects. The before product is deemed to be secure, organic, cost-effective, and environmentally sustainable.[36]

Among the various methods of synthesis, the green synthesis approach is regarded as the most advantageous and secure means. The extant body of literature suggests that a wide range of metals, including but not limited to gold, silver, and selenium, are capable of generating nanoparticles.[37]

Gold and silver were used in the first prepared NPs. NPs made from metalloids are now also being created, and they have several useful qualities. There are many sources of metals and metalloids, so choosing the one that is least dangerous or toxic at all is a crucial task. Selenium (Se) is chosen because the human body needs it and because of its beneficial properties. It possesses numerous beneficial qualities that pertain to various spheres of life.[38]

This study effectively synthesized selenium nanoparticles from the drumstick plant, Moringa oleifera. With the help of dried leaf extract from Moringa oleifera, Selenium nanoparticles were created. SeNPs can be synthesized using a variety of processes, however green synthesis is the most practical, affordable, non-toxic, and environmentally beneficial option. As a result, green synthesis is used. SeNPs were effectively synthesized in the greatest possible quantity under optimal temperature, salt concentration, and pH conditions. DLS, SEM, UV-Visible spectrometry, FT-IR, TLC analysis, ANOVA, bacterial culture, and DPPH assay were the methods used to describe SeNPs. The DLS found that the average size of the extracts at different concentrations was 348 nm. SeNPs had a polydisperse nature and shown antibacterial action against bacteria from soil samples. SeNPs can also be used to treat and/ or prevent diseases brought on by free radicals because of their mild to moderate antioxidant properties, which allow them to be given in place of antibiotics or in combination with them. Therefore, based on all the experimentation and considerations it was determined that the

optimized concentration for the SeNPs formation was found to be 3% with stirring time of 45 mins.

## 5. REFERENCES


1. Baig N., Kammakakam I., Falath W. Nanomaterials: A review of synthesis methods, properties, recent progress, and challenges. Mater. Adv, 2021; 2: 1821–1871. doi: 10.1039/D0MA00807A.
2. Prasad R.D., Charmode N., Shrivastav O.P., Prasad S.R., Moghe A., Sarvalkar P.D., Prasad N.R. A review on concept of nanotechnology in veterinary medicine. ES Food Agrofor, 2021; 4: 28–60. doi: 10.30919/esfaf481.
3. Lateef A., Darwesh O.M., Matter I.A. Microbial Nanobiotechnology. Springer; Singapore: Microbial nanobiotechnology: The melting pot of microbiology, microbial technology and nanotechnology, 2021; 1–19.
4. Mansor N.I., Nordin N., Mohamed F., Ling K.H., Rosli R., Hassan Z. Crossing the blood-brain barrier: A review on drug delivery strategies for treatment of the central nervous system diseases. Curr. Drug Deliv, 2019; 16: 698–711. doi: 10.2174/1567201816666190828153017.
5. Mughal T.A., Ali S., Hassan A., Kazmi S.A.R., Saleem M.Z., Shakir H.A., Nazer S., Farooq M.A., Awan M.Z., Khan M.A., et al. Phytochemical screening, antimicrobial activity, in vitro and in vivo antioxidant activity of Berberis lycium Royle root bark extract. Braz. J. Biol, 2021; 84 doi: 10.1590/1519-6984.249742.
6. Bonifácio B.V., da Silva P.B., dos Santos Ramos M.A., Negri K.M.S., Bauab T.M., Chorilli M. Nanotechnology-based drug delivery systems and herbal medicines: A review. Int. J. Nanomed, 2014; 9: 1–15.
7. Astruc D. Introduction to Nanomedicine. Molecules, 2016; 21: 4. doi: 10.3390/molecules21010004.
8. Peer D., Karp J.M., Hong S., Farokhzad O.C., Margalit R., Langer R. Nanocarriers as an emerging platform for cancer therapy. Nano-Enabled Med. Appl, 2021; 61–91. doi: 10.1201/9780429399039-2.
9. Gurunathan S., Kalishwaralal K., Vaidyanathan R., Venkataraman D., Pandian S.R.K., Muniyandi J., Hariharan N., Eom S.H. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf. B Biointerfaces, 2009; 74: 328–335. doi: 10.1016/j.colsurfb.2009.07.048.

10. Faramarzi S., Anzabi Y., Jafarizadeh-Malmiri H. Nanobiotechnology approach in intracellular selenium nanoparticle synthesis using Saccharomyces cerevisiae—Fabrication and characterization. Arch. Microbiol, 2020; 202: 1203–1209. doi: 10.1007/s00203-020-01831-0.
11. Sweeney R.Y., Mao C., Gao X., Burt J.L., Belcher A.M., Georgiou G., Iverson B.L. Bacterial Biosynthesis of Cadmium Sulfide Nanocrystals. Chem. Biol, 2004; 11: 1553–1559. doi: 10.1016/j.chembiol.2004.08.022.
12. Bai H.J., Zhang Z.M., Guo Y., Yang G.E. Biosynthesis of cadmium sulfide nanoparticles by photosynthetic bacteria Rhodopseudomonas palustris. Colloids Surf. B Biointerfaces, 2009; 70: 142–146. doi: 10.1016/j.colsurfb.2008.12.025.
13. Pottathara Y.B., Grohens Y., Kokol V., Kalarikkal N., Thomas S. Chapter 1—Synthesis and Processing of Emerging Two-Dimensional Nanomaterials. In: Beeran Pottathara Y., Thomas S., Kalarikkal N., Grohens Y., Kokol V., editors. Nanomaterials Synthesis. Elsevier; Amsterdam, The Netherlands, 2019; 1–25.
14. Jain PK, ElSayed IH, El-Sayed MA. Au nanoparticles target cancer. Nano Today, 2007; 2(1): 18-29.
15. Anderson SD, Gwenin V V., Gwenin CD. Magnetic Functionalized Nanoparticles for Biomedical, Drug Delivery and Imaging Applications. Nanoscale Res Lett, 2019; 14(1): 1-16.
16. Sharma A, Goyal AK, Rath G. Recent advances in metal nanoparticles in cancer therapy. J Drug Target, 2018; 26(8): 617-632.
17. Malekzad H, Sahandi Zangabad P, Mirshekari H, Karimi M, Hamblin MR. Noble metal nanoparticles in biosensors: recent studies and applications. Nanotechnol Rev, 2017; 6(3): 301-329.
18. M. Zhu, G. Niu and J. Tang, J. Mater. Chem. C, 2019; 7: 2199–2206.
19. J. F. Ramos and T. J. Webster, Int. J. Nanomed, 2012; 7: 3907–3914.
20. J. E. Spallholz, Free Radic. Biol. Med, 1994; 17: 45–64.
21. F. Sieber, J.-P. Daziano, W. H. H. Günther, M. Krieg, K. Miyagi, R. W. Sampson, M. D. Ostrowski, G. S. Anderson, I. Tsujino and R. J. Bula, Phosphorus Sulfur Silicon Relat. Elem, 2005; 180: 647–657.
22. M. Vahdati and T. Tohidi Moghadam, Sci. Rep, 2020; 10: 510.
23. H. Forootanfar, M. Adeli-Sardou, M. Nikkhoo, M. Mehrabani, B. Amir-Heidari, A. R. Shahverdi and M. Shakibaie, J. Trace Elem. Med. Biol, 2014; 28: 75–79.

24. M. Panahi-Kalamuei, M. Salavati-Niasari and S. M. Hosseinpour-Mashkani, J. Alloys Compd, 2014; 617: 627–632.
25. J.-S. Zhang, X.-Y. Gao, L.-D. Zhang and Y.-P. Bao, BioFactors, 2001; 15: 27–38.
26. J. Zhang, X. Wang and T. Xu, Toxicol. Sci, 2008; 101: 22–31.
27. P. A. Tran and T. J. Webster, Int. J. Nanomed, 2011; 6: 1553.
28. P. A. Tran and T. J. Webster, Nanotechnology, 2013; 24: 155101.
29. Q. Wang and T. J. Webster, J. Biomed. Mater. Res., Part A, 2012; 100: 3205–3210.
30. A. Kumar and K. S. Prasad, J. Biotechnol, 2021; 325: 152–163.
31. M. Ikram, B. Javed, N. I. Raja and Z. U. R. Mashwani, Int. J. Nanomed., 2021; 16: 249–268.
32. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants (Basel), 2019; 11, 8(4): 96. doi: 10.3390/plants8040096. PMID: 30978964; PMCID: PMC6524357.
33. Cittrarasu V, Kaliannan D, Dharman K, Maluventhen V, Easwaran M, Liu WC, Balasubramanian B, Arumugam M. Green synthesis of selenium nanoparticles mediated from Ceropegia bulbosa Roxb extract and its cytotoxicity, antimicrobial, mosquitocidal and photocatalytic activities. Sci Rep, 2021; 13, 11(1): 1032. doi: 10.1038/s41598-020-80327-9. PMID: 33441811; PMCID: PMC7806947.
34. MacLeod, R.A., Onofrey, E., Norris, M.E., Nutrition and metabolism of marine bacteria I.: survey of nutritional requirements. J Bacteriol, 1954; 68(6): 680.
35. Bootz A, Vogel V, Schubert D, Kreuter J. Comparison of scanning electron microscopy, dynamic light scattering and analytical ultracentrifugation for the sizing of poly (butyl cyanoacrylate) nanoparticles. European Journal of Pharmaceutics and Biopharmaceutics, 2004; 57: 369–75.
36. Tran PA, Webster TJ., Selenium nanoparticles inhibit Staphylococcus aureus growth. Int J Nanomedicine, 2011; 6: 1553–8.
37. https://rtilab.com/techniques/ftir-analysis/.
38. Paliwal, R., Sharma, V., & Sharma, S. Elucidation of free radical scavenging and antioxidant activity of aqueous and hydro-ethanolic extracts of Moringa oleifera pods. Research Journal of Pharmacy and Technology, 2011; 4(4): 566-571.
39. https://byjus.com/chemistry/thin-layer-chromatography/#Procedure.
40. https://www.investopedia.com/terms/a/anova.asp.

41. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. Molecules, 2022; 16, 27(4): 1326. doi: 10.3390/molecules27041326. PMID: 35209118; PMCID: PMC8878429.