Silver Nanoparticles of Moringa oleifera - A Comprehensive Study on Antioxidant, Anti-Inflammatory and Cell Line Based Anticancer Properties

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Abstract
Silver nanoparticles (AgNPs) have gained considerable attention as potential therapeutic agents owing to their notable antioxidant, anti-inflammatory, and antitumor activities. The present study focuses on the eco-friendly synthesis of AgNPs utilizing *Moringa oleifera* flower extract and explores their biological properties. The formation of AgNPs was confirmed by UV–visible spectroscopy, which exhibited a characteristic surface plasmon resonance peak at 430 nm. The antioxidant potential of the synthesized AgNPs was evaluated using standard in vitro assays, demonstrating effective free radical scavenging activity with a maximum inhibition of 54.63 % inhibition at a concentration of 1000µg/ml, indicating their ability to mitigate oxidative stress implicated in chronic disorders such as diabetes and cardiovascular diseases. Additionally, the AgNPs exhibited significant anti-inflammatory activity, achieving 64.70 % inhibition at 100µg/ml, suggesting their therapeutic relevance in inflammatory conditions. The anticancer activity of the synthesized AgNPs was evaluated using the MTT assay against the MCF-7 human breast cancer cell line. The results, based on absorbance measurements, revealed a concentration-dependent effect on cell viability. At the lowest concentration tested (7.8 µg/ml), MCF-7 cells exhibited higher cell viability (63.37%), indicating minimal cytotoxicity. However, with increasing concentrations of AgNPs (1000µg/ml), a gradual decrease in absorbance and cell viability (31.42%) was observed, reflecting reduced metabolic activity and enhanced cytotoxic effects. These findings provide a valuable foundation for future preclinical investigations and suggest that green-synthesized AgNPs could contribute to the development of novel therapeutic strategies for cancer and other chronic diseases.

Keywords: Inflammation, oxidative stress, silver nanoparticles (AgNPs), *Moringa oleifera* flower, Green synthesis, Breast cancer.

Introduction

The development of cancer often results in the loss of healthy cells, which in turn causes various toxic

effects and side effects in patients**.** Therefore, it is there is an urgent need to find novel and effective alternatives to cure this disease [1]. Silver nanoparticles (AgNPs) have been used as a popular antimicrobial agent for quite a long time [2]. Investigations were carried out on A549, MCF-7 (Michigan Cancer Foundation-7), A431, and HeLa cells, and 50% cell death in MCF-7 after 48 hours of treatment with AgNPs synthesized from *Cineraria prosposis* leaf extract was reported [3]. The effects were found to be dose-responsive. The effectiveness of silver nanoparticles in killing cancer cells was observed only at higher doses [4, 5].
Phytochemicals have more recently emerged as key modulators of cellular signalling pathways [6]. Recent studies have shown that many phytochemicals can benefit the function of human cells [7]. Major classes of phytochemicals include alkaloids, flavonoids, tannins, phenolics, saponins, steroids, glycosides, and terpenes. Furthermore, when consumed in sufficient dietary amounts, these compounds can contribute to the prevention of various human diseases [8].
Oxidative stress occurs when the body's antioxidant defense system fails to neutralize an excess of reactive oxygen species (ROS). Research has shown that oxidative stress-induced free radicals play a significant role in aging and the development of numerous diseases, including neurodegenerative disorders, chronic inflammation, cardiovascular diseases, and cancer [9]. Cells are equipped with various mechanisms to combat ROS and maintain cellular redox homeostasis [10].
In response to tissue damage, inflammatory cells are recruited to the affected site, where a cascade of signalling molecules—including proinflammatory cytokines such as interleukins (IL-1, IL-6, IL-12, IL-18), tumour necrosis factor (TNF), interferon-gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF)—are released [11]. Various plant parts—including leaves, flowers, roots, gums, and fruits—have been widely used in traditional medicine to treat inflammation [12].
The flowers of *Moringa oleifera* are rich in calcium, potassium, and antioxidants such as α- and γ-tocopherol, and are commonly included in the human diet, particularly in the Philippines [13]. In addition, the flowers have been reported to exhibit antitumor properties [14]. Previous reports have also highlighted the plant's strong anti-inflammatory [15] and antitumor activities [16]. The aim of the present study is to investigate the effect of *Moringa oleifera* flower fortification on the nutritional quality and also to investigate the antioxidant, anti-inflammatory and anticancer properties.

Materials and Methods

Plant Collection and Sample Preparation

The *Moringa oleifera* flowers were collected from the garden and transported to the laboratory in aseptic condition. The flowers were cut into pieces and shade dried for ten days of time. After drying the sample were powered using mortar and pestle stored in air tight container at room temperature.

Procedure

Twenty-five grams of the powdered sample were weighed and kept inside the thimble. The sample loaded thimble was placed into the Soxhlet apparatus and extracted with ethanol solvent for ten hours of time. The extracts were condensed using rotary evaporator at 50˚C under reduced pressure. After condensation the samples were reconstituted in ethanol solvents to obtain a stock of 100 mg/ml and were stored in a refrigerator for further study [17].

Phytochemical Analysis

Test for Tannins

1ml of the ethanolic extract was mixed with two drops of 0.1 % ferric chloride. Appearance of brownish green or blue-black coloration was found to be positive for tannins.

Test for Saponins

To 1 ml of the extract two ml of water was added and shaken for fifteen seconds of time. Formation of foam indicates the presence of saponin in the sample.

Test for Flavonoids

Few drops of sodium hydroxide were added to 1 ml of the extract and observe the formation of yellow colour and then to white colour when few drops of hydrochloric acid were added.

Test for Alkaloids

1 ml of the extract was mixed with two to three drops of dragendorff reagent. Appearance of prominent yellow precipitate confirms the presence of alkaloids in the sample.

Test for Proteins

Few drops of Bradford's reagent were mixed with 1 ml of the extract and observed for the greenish blue colouration. The change in colour confirms the presence of protein in the sample.

Test for Steroids

Appearance of brown colour is found to be positive when two drops of 10% concentrated sulphuric acid was added to 1 ml of sample.

Test for Anthraquinones

1 ml of the extract was taken, to those five drops of ammonia solution was added. Appearance of Pink, red, or violet colour in aqueous layer confirms the presence of anthraquinones.

Test for Phenols

1ml of extract was mixed with 3ml of 10% Lead acetate solution. A bulk white precipitate formed at the surface indicates the presence of phenolic compounds.

Test for Terpenoids

To one ml of the sample 2 ml of chloroform followed by 3 ml of concentrated sulphuric acid was added. Formation of red brown colour at the interface confirms the presence of terpenoids.

Test for Carbohydrates

To one ml of the sample 0.5ml of Benedict's reagent was added. Formation of blue coloured precipitate indicates the presence of carbohydrates. [18].

Silver Nanoparticles Synthesis

Synthesis of silver nanoparticles were executed using the reduction method. To synthesise silver nanoparticle approximately ten ml each of organic and aqueous extract was mixed with 30 ml of 1mM silver nitrate solution in an Erlenmeyer flask. The mixture is kept under vigorous shaking (500 rpm) at 450C for 2 hours using magnetic stirrer followed by incubation at room temperature for 48-72hours of time in dark to protect from sunlight. During incubation colour change was monitored. While the process is going on a progressive colour change from yellow to dark brown reveals the production of silver nanoparticles [19].

UV –Vis Spectroscopy

The reduction of silver nitrate and the formation of nanoparticle was monitored by UV-VIS systronics spectrophotometer in 350-500nm range [20]. The nanoparticle mixture was centrifuged at 10000 rpm for fifteen minutes to separate the silver nanoparticles. After centrifugation the pellets obtained was collected, lyophilized and characterised by various techniques [21].

Determination of Antioxidant Activityof Ethanol Flower Extract of *Moringa oleifera*

Dpph (1,1-Diphenyl-2-Picrylhydrazyl) Assay

Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. Add 100µl of BHT (Butylated Hydroxy Toluene) to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm. [22].

% Antioxidant activity = (Absorbance at blank) – (Absorbance at test) x100
(Absorbance at blank)

Determination of Anti Inflammatory Activity of Ethanol Flower Extract of *Moringa oleifera*

Inhibition of Protein Denaturation

100µl of test was added with 500µl of 1% BSA (Bovine Serum Albumin). The mixture was incubated for 10 minutes at 37ºC. Heat the contents in a water bath at 51ºC for 20 minutes. Cool down to room temperature and check the absorbance at 660nm against the blank. Acetyl Salicylic acid was used as positive control and water as product control [23].

%Inhibition = (Control O.D – Sample O.D) x100
Control O.D

Determination of Anticancer Activity of Ethanol Flower Extract of *Moringa oleifera*

Cell line and culture

MCF 7 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM (Dulbecco's Modified Eagle Medium**)** supplemented with 10% FBS (Fetal Bovine Serum**)**, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

In Vitro assay for Anticancer activity: (MTT assay)

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37^0C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO (dimethyl sulfoxide) was added in all the wells. The absorbance at 570nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically [24]. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells
A570 Cell line and culture

Result and Discussion

Phytochemicals Analysis

Pharmacological studies have shown that *M. oleifera* possesses various therapeutic properties, including hypoglycemic, hypotensive, antimicrobial, hepatoprotective, immunomodulatory, antioxidant, and antitumor activities [25, 26, and 27]. These bioactivities are largely attributed to the presence of secondary metabolites such as carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolic compounds [28]. Besides tannins and phenolic compounds, the plants also contained alkaloids, saponins, and flavonoids. Flavonoids are water-soluble antioxidants and free radical scavengers that protect against oxidative damage and show strong anticancer activity [29, 30, and 31]. This study identified the presence of various phytochemicals, including alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenoids, and carbohydrates, which are attributed to the observed antioxidant, anti-inflammatory, and anticancer activities of the ethanol flower extract of *Moringa oleifera* (Tab 1).

Table 1: Result of Phytochemical Analysis of Ethanol Flower Extract of Moringa oleifera

TEST	RESULT
Alkaloids	++++
Flavonoids	++++
Tannins	++++
Saponins	++++
Steroids	++++
Phenol	++++
Protein	++++
Anthraquinones	----
Terpenoids	++++
Carbohydrates	++++
++++: Presence of constituents ----: Absence of constituents	

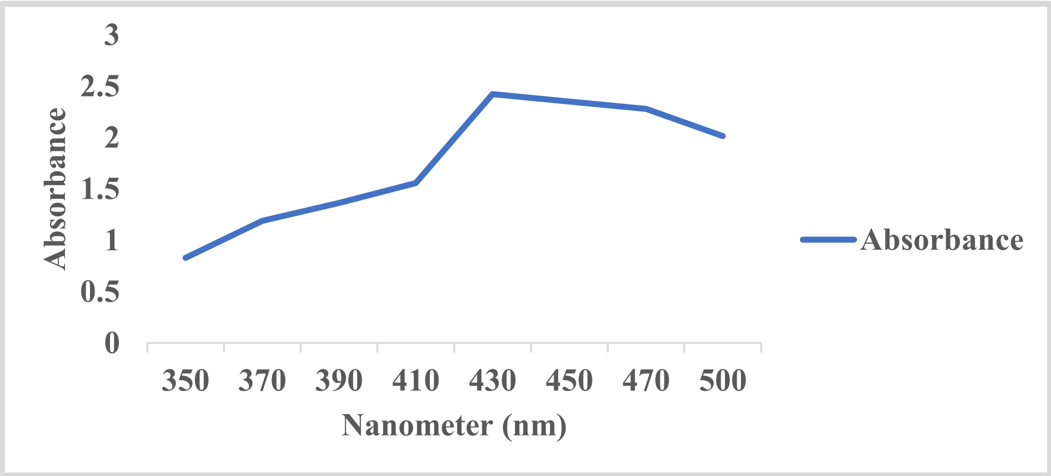
Silver Nanoparticles Synthesis

The synthesis of silver nanoparticles (AgNPs) by *Moringa oleifera* stem bark extract was observed upon incubation with $AgNO_3$ solution. A gradual color change from yellow to reddish brown was observed, corresponding to the surface plasmon resonance (SPR) of AgNPs. No further color change after reduction indicated nanoparticle stability. This suggests the presence of reducing biomolecules responsible for silver ion reduction. The color change correlated with concentration and temperature, likely due to reducing agents such as terpenoids, flavonoids, and polysaccharides. Additionally, nanoparticles appeared to interact with plant proteins via free amine groups or cysteine residues. Aggregation observed under AFM (Atomic Force Microscopy) may have resulted from sample preparation. The synthesized AgNPs carried a negative charge due to adsorbed flavonoids and phenols, which prevented aggregation through electrostatic stabilization [32]. Furthermore, certain bio-organics in the *M. oleifera* flower extract may have contributed to electrosteric stabilization. The optical density of the synthesised nanoparticle has gradually increased and attend its optimum peak (430nm) and decreased gradually as shown (Tab 2). The graph (Fig 1) clarify the presence of nanoparticle.

Table 2: Result of Presence of Silver Nanoparticles of Ethanol Flower Extract of Moringa oleifera

Nano meter	Absorbance
350nm	0.829
370nm	1.191
390nm	1.364
410nm	1.558
430nm	2.423
450nm	2.351
470nm	2.278
500nm	2.014

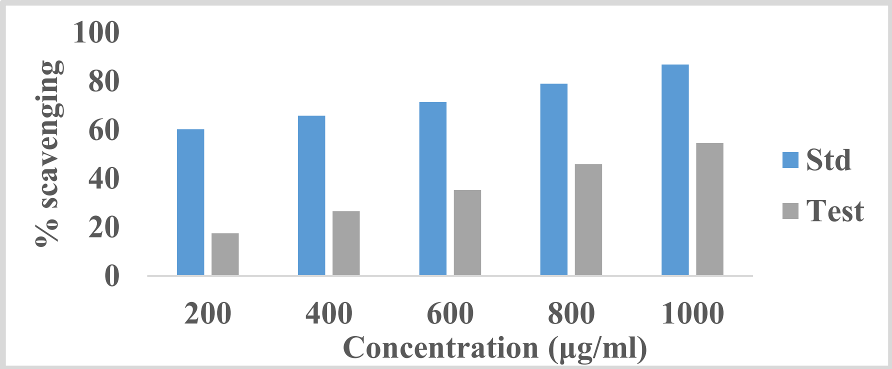
Figure 1: Graphical Representation of Silver Nanoparticle of Moringa oleifera



Antioxidant Activity of Ethanol Flower Extract of *Moringa oleifera*

The strong antioxidant activity of *Moringa oleifera* is attributed to its high polyphenol content. The antioxidant potential of biosynthesized AgNPs is attributed to their high phenolic content, as determined by the DPPH radical scavenging assay [33]. Capped silver nanoparticles demonstrated strong free radical scavenging activity. The antioxidant activity of AgNPs may also be attributed to the functional groups present on their surface [34]. Several studies have reported that phenolic compounds in plant extracts play a key role in conferring both antioxidant and antimicrobial properties to nanoparticles [35]. Various methods are employed to evaluate the antioxidant activity of metal nanoparticles, with the DPPH assay being the most widely used due to its reliability in assessing free radical scavenging capacity. The assay is based on the hydrogen- or electron-donating ability of antioxidant agents. DPPH results demonstrated a dose-dependent increase in antioxidant activity [36]. The IC_{50} value against DPPH free radicals was higher than that of ascorbic acid (27.70 µg/ml vs. 55.0 µg/ml, respectively), indicating that the extract can provide a measurable degree of antioxidant activity when consumed in sufficient quantities [37]. In present study shows that the IC50 value for the antioxidant activity of synthesized silver nanoparticles in *Moringa oleifera* flower extract, using ethanol as the solvent, was 572.72µg/ml. In comparison, BHT (Butylated Hydroxy Toluene) had an IC50 value of 606.2µg/ml, indicating it has stronger antioxidant activity at a lower concentration. The result of the present study induced silver nanoparticles in *Moringa oleifera* ethanol extract reveals that has antioxidant inhibition is shown in the bar graph (Fig 2).

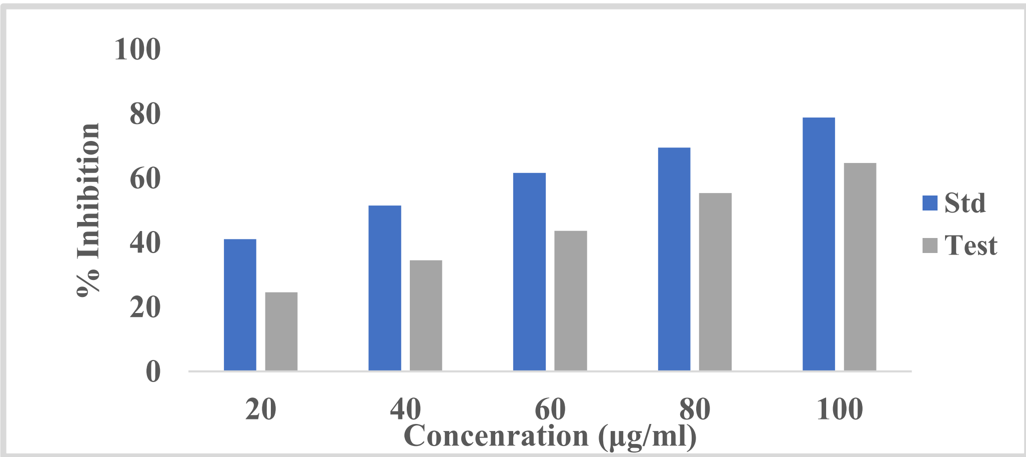
Figure 2: Graphical Representation of Antioxidant Activity of Moringa oleifera



Anti-Inflammatory Activity of Ethanol Flower Extract of *Moringa oleifera*

The effect of *Moringa oleifera* hexane extract on suppressing the release of nitric oxide (NO), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)—key mediators in the pathogenesis of inflammation—was evaluated in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. Compared with the LPS-stimulated control, *Moringa* hexane extract and celecoxib significantly reduced NO production by 30% and 68.6%, respectively. Similarly, the release of IL-6 was reduced by 46.8% with *Moringa* extract and 80.2% with celecoxib, while TNF-α levels decreased by 74.7% and 91.3%, respectively. Although celecoxib, a non-steroidal anti- inflammatory drug, demonstrated higher efficacy than *Moringa* extract, the latter exhibited a broader safety margin with moderate anti-inflammatory activity. These findings are consistent with previous reports on the anti-inflammatory properties of *Moringa* flowers, though those studies employed alcoholic extracts [38, 39]. Nitric oxide, a labile free radical gas, is a crucial mediator and regulator of inflammatory responses and is excessively generated during inflammation [40]. The hexane extract did not exhibit significant cytotoxicity at 1 µg/ml, with an IC_{50} value of 398.53 µg/ml, compared to celecoxib, which showed an IC_{50} value of 274.55 µg/ml [41]. The IC50 value for the anti-inflammatory activity of synthesized silver nanoparticles in *Moringa oleifera* flower extract, using ethanol as the solvent, was 59.65 µg/ml. In comparison, acetylsalicylic acid (ASA) had an IC50 value of 60.48 µg/ml, showing similar anti-inflammatory effects. The result of the present study induced silver nanoparticles in *Moringa oleifera* ethanol extract reveals that has anti-inflammatory inhibition is shown in the bar graph (Fig 3).

Figure 3: Graphical Representation of Anti-Inflammatory Activity of Moringa oleifera



Anticancer Activity of Ethanol Flower Extract of *Moringa oleifera*

Silver nanoparticles, in particular, are widely recognized for their antimicrobial and antioxidant activities, along with relatively low toxicity [42]. In this study, the MTT assay was chosen to assess anticancer activity in order to avoid interference from cross-coloration between nanoparticles and other dissolving dyes. In this assay, formazan crystals form exclusively in viable cells and dissolve in dimethyl sulfoxide (DMSO), producing a blue-colored product. that can be quantified spectrophotometrically. The absence of formazan formation in dead cells allows for precise evaluation of cell viability. Exposure of cancer cell lines to AgNPs resulted in sub-G1 cell cycle arrest and apoptosis. Moreover, silver nanoparticles have been reported to inhibit tumor cell migration and angiogenesis, thereby potentially reducing distant metastasis [43,44]. (Table 3) presents the cell viability data for silver nanoparticles synthesized from the ethanol extract of *Moringa oleifera*, while (Figures 4 and 5) illustrate the corresponding graphical representation of cell viability at different concentrations.

Table 3: Result Of Anticancer Activity of Ethanol Flower Extract of Moringa oleifera - Anticancer effect of Sample on MCF 7 cell line for 24 hours

S.No	Concentration (µg/ml)	Absorbance (O.D)	% Cell viability
1	1000	0.236	31.42
2	500	0.273	36.35
3	250	0.311	41.41
4	125	0.349	46.47
5	62.5	0.384	51.13
6	31.2	0.434	57.78
7	15.6	0.477	63.51
8	7.8	0.521	69.37
Cell control		0.751	100

Figure 4: Graphical Representation of Anticancer Activity of Moringa oleifera

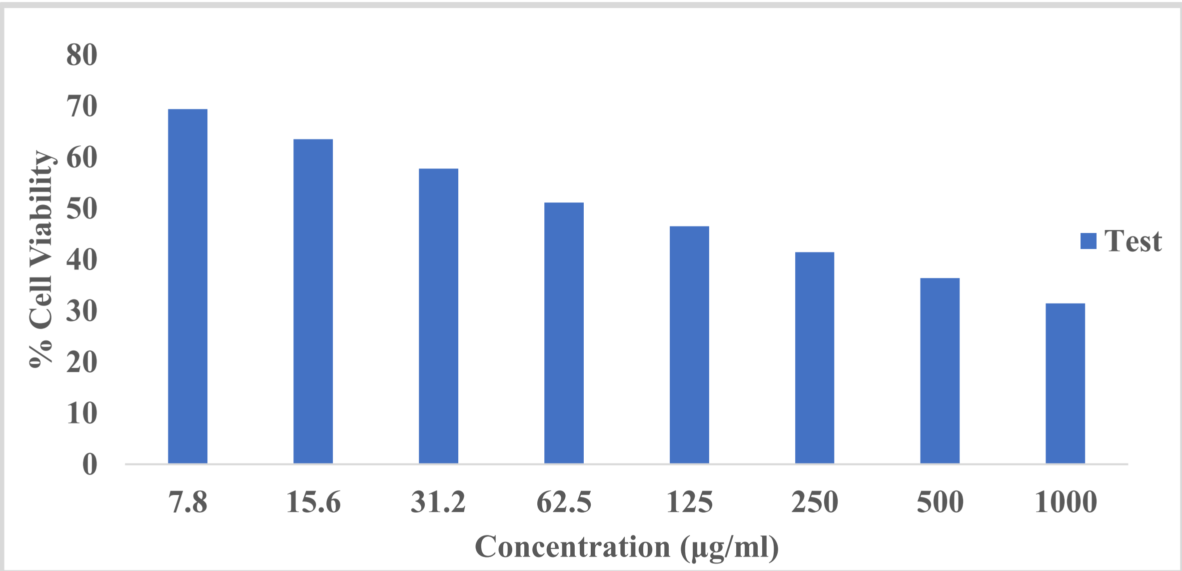
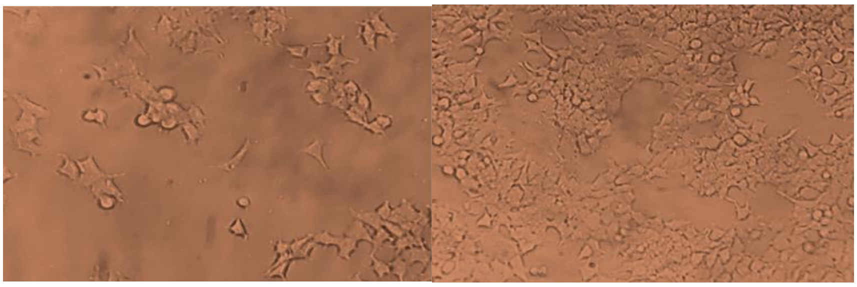
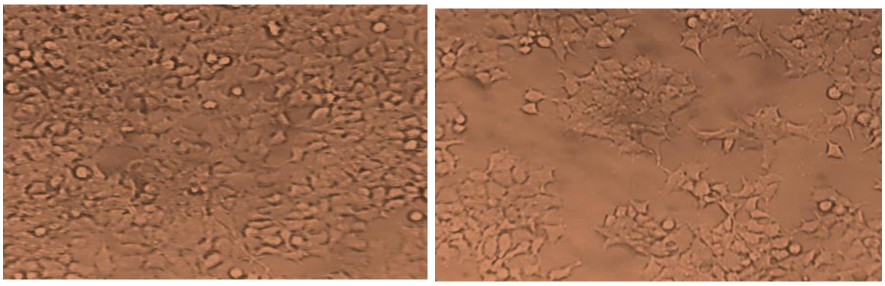


Figure 5: Cell Viability of Ethanol Extract of Moringa oleifera in Different Concentration a. Control Cell b. 62.5 µg/ml c.1000 µg/ml d. 7.8 µg/ml of Ethanol Extract of Moringa oleifera

a. control cell **b. 62.5 µg/ml**



c. 1000 µg/ml **d.7.8 µg/ml**



Conclusion

This study investigated the potential of *Moringa oleifera* flowers in the synthesis of silver nanoparticles (AgNPs) and evaluated their biological activities, including phytochemical, antioxidant, anti-inflammatory, and anticancer properties. *Moringa oleifera* flowers, which contribute to its diverse therapeutic effects. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenoids, and carbohydrates, with flavonoids and phenolic acids. The biosynthesized AgNPs exhibited strong antioxidant activity by effectively scavenging free radicals and mitigating oxidative stress. They also demonstrated significant anti-inflammatory activity. Furthermore, anticancer activity was confirmed via the MTT assay, which revealed a dose-dependent cytotoxic effect on selected cancer cell lines, indicating the nanoparticles' ability to inhibit tumor cell growth.

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