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GREEN SYNTHESIS OF SELENIUM NANOPARTICLES FROM MULBERRY LEAVES AND ITS CHARACTERIZATION

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ABSTRACT

Selenium is a crucial trace element and the building block of many proteins. Humans who are deficient in selenium are more susceptible to myodegenerative disease, cardiovascular disease, and other illnesses. A small amount of selenium is required by the body to treat several disorders. Nano-selenium's easy cell penetration gives it a more powerful effect. When compared to chemically produced nanoparticles, biologically produced nanoparticles displayed lower toxicity. Mulberries are a rich source of vitamin C and iron, and previous studies have shown that they have a variety of pharmacological properties that include effects that are anti-inflammatory, anti-obesity, anti-hypertensive, anti-oxidative, anti-cancer, anti-atherosclerotic, and cardioprotective. In this study, nanoparticles were synthesized from mulberry leaves and characterization of selenium nanoparticles was done using analytical methods such ultraviolet-visible spectroscopy, scanning electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction. These selenium nanoparticles were assessed for biological activities such as antioxidant, antimicrobial, and antitumor effects. Results demonstrated that selenium nanoparticle synthesized from mulberry showed particle size of 397nm in Scanning electron microscope analysis. In X- ray diffraction, nanoparticles showed broad peak at 2Θ angle of 20-250 which means it is crystalline in nature. Fourier transform infrared spectroscopy analysis confirmed presence of alcohols, carbonyl group, methyl group, aldehyde group, esters, amine, ketones, amines were responsible for the formation selenium nanoparticles from mulberry leaves. The selenium nanoparticles exhibited maximum antioxidant activity in 2,2-Diphenyl-1-picryl-hydrazyl-hydrateassay were 48.57µg/ml when compared to control was 46µg/ml. In 2,2′-azino-bis3-ethylbenzothiazoline-6-sulfonic acid assays, IC50 of control was 49.3µg/ml and sample were 48.08µg/ml. SeNP showed IC50 of 49.08µg/ml and control were 43.5µg/ml in Nitric Oxide assay. In ferric ion reducing antioxidant power, IC50 value of nanoparticles was 41.069µg/ml and control were 41.54µg/ml. Maximum antifungal activity against *Aspergillus niger* at a concentration of 400µg and antibacterial activity against *Staphylococcus aureus* and *Streptococcus mutans* was at 500 µg of concentration was observed. Utmost antitumor activity was observed in 25µl of nanoparticles with an inhibition percentage of 87.96% against MCF-7 (breast cancer cell lines). The main objective of this study is to synthesize selenium nanoparticles by biological method. These nanoparticles can be effectively used in the drug delivery system to increase the bioavailability and efficacy of the drug molecule against various diseases.

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INTRODUCTION

Selenium, red colored amorphous powder is an essential trace element. Selenium salts such as selenite and selenates in the presence of reducing agents like proteins, phenols and amines produce different sizes and shapes of selenium nanoparticles(1). The four inorganic chemical forms of selenium present in natural environment are selenite (SeO_4^{-2}), selenite (SeO_3^{-2}), elemental selenium (Se^0) and selenide (Se^{-2}). Twenty-five selenoproteins such as Thioredoxin reductases, Glutathione peroxidases, Thyroid hormone deiodinases, selenoprotein P, Sep 15, selenoprotein N, selenoprotein W, selenophosphate synthetase 2, selenoprotein R, selenoprotein M, selenoprotein S, selenoprotein K etc. selenium play an important role in human body (1). Selenium enters animals via plants. Plants uptake inorganic form of selenium from soil and converts it into organic forms such as Selenomethionine (SeMet), which is the main seleno compound in grains, legumes and soya beans. In animals, Selenomethionine is the precursor of selenocysteine (Sec) which is the active form of selenium and also an analogue of Cys used to produce antioxidant glutathione which in turn makes DNA and also supports immune function(3).

Selenium nanoparticles can be produced in three ways such as physical, chemical and biological methods (figure 1). Physical methods mainly involve high temperature spraying method (4), laser ablation (5). Chemical methods involve reduction of sodium selenite (6).

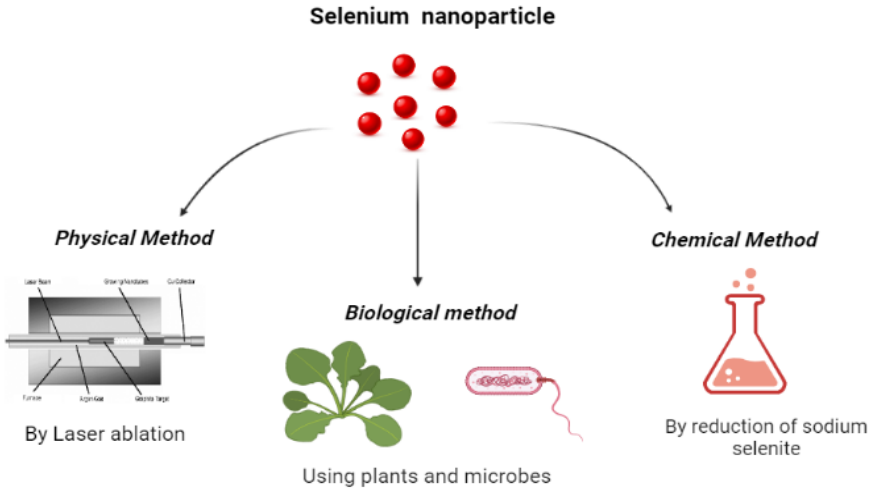

Figure 1: Image showing the different ways of nanoparticle synthesis. Image was created using biorender.com.

Many microbes were used in the production of selenium nanoparticles such as *Lactobacillus casei, Streptococcus thermophilus, and Klebsiella pneumonia*(7), *Escherichia. Coli* (8),*Veillonella atypica* (9), *Pseudomonas Spp*(10).

Some plants were also used in the production of selenium nanoparticles such as *Vitis vinifera* (11), *Capsicum annum*(12),Broccoli(13), Parsley leaves extract (14), *Allium sativum*(15,16),Fenugreek seed extract (17),Tea extract(18), *Clausena dentana* plant leaf extract (19), *Undaria pinnatifida* polysaccharide solutions (20), *Terminalia arjuna* leaves (21), *Leucas lavandulifolia*(22) and some medicinal plants namely plantain (*Plantago lanceolata L.),* yarrow (*Achilea millefolium L.)* and nettle (*Urtica dioica L.*) (23).

Deficiency of selenium also leads to many diseases in humans such as increased cancer risk, decrease in immunity and thyroid function, Alzheimer disease, Parkinson diseases (24) and rheumatoid arthritis(25). Size of the selenium nanoparticle plays a main role in showing its antioxidant activity such as smaller selenium nanoparticles show better activity when compared with the larger particles(26). Selenium nanoparticles have many applications related to Anti-tumor activity(27), immunomodulatory effect, Anti-inflammatory activity(28).Drugs loaded with selenium nanoparticles alleviated cancer cells by suppressing their proliferation (29).It's interesting to note that many medicinal plants have long been utilized as nutritional supplements and complementary medications for the treatment and prevention of many diseases. The possible source of many bioactive chemicals is believed to be plants. Green synthesized selenium nanoparticles from plant materials played a beneficial role in promoting plant growth metabolism, stress tolerance and thereby increasing the biofortification(30). Plant extracts from leaves of amphipteygium glaucum and flowers of calendula officinalis were used to synthesize selenium nanoparticles. Different concentrations of these Se-NP's showed antifungal activity against plant pathogenic fungi namely *Fusarium oxysporum* and *Colletotrichum gloeosporioides*(31). Selenium nanoparticles also stimulated osteoblast differentiation in diabetic osteoporosis condition (31).

Based on the previous literatures, selenium nanoparticles have wide applications in therapeutics. Mulberry is a plant with several uses. Mulberry has been recognized as a functional food due to its strong nutritional and phytochemical content (33). Nanoparticles have also been used for food packing, surface protection, textiles, energy production, and waste-water treatment etc., As many drugs are available in the market for various diseases, but using nanoparticles tagged with drugs increases the efficacy and accurate delivery of the drug. Since only few works have been done on green synthesis of selenium nanoparticles using mulberry leaves as a source. The present study focused to evaluate the best variety of mulberry leaves for the synthesis of selenium nanoparticles (SeNP) and also determine their biological applications.

MATERIALS AND METHODS

Synthesis of selenium nanoparticles by using varieties of mulberry leaves

Production of mulberry leaves extract:

Five different mulberry varieties named Suvarna1, Suvarna 2, V_1, Vishala and M_5were collected from Karnataka state sericulture research and development institute, Bangalore and labelled as 1, 2, 3,4 and5. All the samples were dried and powdered. The secondary metabolites were extracted from the leaves through Soxhlet extraction using methanol as the solvent. The solution obtained was further distilled and evaporated to dryness for further use.

Synthesis of selenium nanoparticles using mulberry leaves extract:

Selenium nanoparticles were synthesized using the method followed by [15]. Spectrum absorbance readings were observed periodically (0hrs, 2hrs, 4hrs and 6hrs) using UV visible spectrophotometer (figure 2).

Production of selenium nanoparticles from mulberry leaves extract:

Bulk production of selenium nanoparticles was done using 5^{th} sample(M_5) which showed highest absorbance at 4hrs of incubation time.'

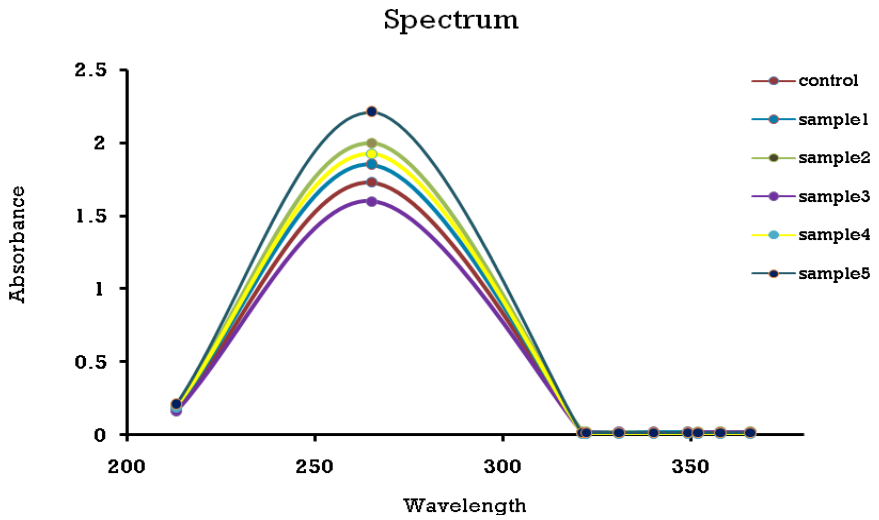

Figure2: Spectrum analysis of 5 types of mulberry leaves nanoparticles at 4 hours of incubation time.

Characterization of selenium nanoparticles

The selenium nanoparticles synthesized were characterized using and SEM, XRD, and FTIR, analysis. Scanning electron microscope (SEM) analysis is mainly done for determining the particle size. Fourier transform infrared (FTIR) analysis is done for identifying the functional groups. X- ray diffraction(XRD) analysis is done for determining the crystallinity of the nanoparticles.

To study biological activities of selenium nanoparticles

Many studies revealed that selenium nanoparticles have many biological activities such as antimicrobial, antioxidant activity and antitumor activity.

Antifungal activity of selenium nanoparticles:

Fungal pathogens such as *Aspergillus Niger*, *Aspergillus flavus,* and *Candida albicans* were cultured for 24hrs and spread onto 3 separate petri-plates containing potato-dextrose agar (potato extract-900ml, dextrose – 20gms, agar-20gms and distilled water 100ml) and 4 wells were made in each of the petri-plate. Selenium nanoparticles (control and sample) were loaded into 4 wells at different concentrations (100µg, 200µg, 300µg and 400µg) and incubated for 24hrs. After incubation, inhibition zones were measured.

Antibacterial activity of selenium nanoparticles:

Selenium nanoparticles (sample and control) were assessed for their MIC property against *Bacillus cereus, Staphylococcus aureus, Streptococcus mutans, E-coli, Salmonella typhi* and *Pseudomonas aeruginosa* by resazurin method using 96 microtiter plate. Initially,water was added to the outermost wells of the 96-microtiter plate. Remaining all the wells were filled with 100µl of sterileLuria Broth (tryptone 10g, sodium chloride 10g, yeast extract 6g and distilled water 1000mL). To this 100µl of Selenium nanoparticles (10mg of extract in 1ml DMSO) were added. Finally, 100µl of Bacillus cereus, Staphylococcus aureus, Streptococcus mutans, E-coli, Salmonella typhi and Pseudomonas aeruginosa and 30µl of resazurin dye were added to all the wells and incubated for 24hrs. Presence of blue color indicates no growth of the organism (inhibited) whereas appearance of pink color indicates growth of the organism (not inhibited).

Antioxidant activity

DPPH assay:

Free radical scavenging capacity of the extracts was estimated using the stable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical. Different concentrations of the sample and control (100µg, 200µg, 300µg, 400µg and 500µg) were taken in the test tubes and the volume was made up to 100µL with methanol and then 3mL of DPPH solution was added and incubated in dark condition for 15minutes. After incubation, the absorbance was read at 517nm spectrophotometrically with methanol as a blank. IC50 value was calculated for selenium nanoparticles (control and sample). The percentage radical scavenging activity of the methanol extracts were calculated using the following formula:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_o = Abs of control, A_1=Abs. of tested samples

ABTS assay:

Four concentrations of selenium nanoparticles (100µg, 200µg, 300µg, 400µg and 500µg) were taken in the test tubes and the volume was made up to 100µL with methanol. To all the tubes, 3mL of ABTS (absorbance is preset to 1 at 734nm) solution was added and incubated in dark condition for30 minutess. After incubation, using methanol as blank the absorbance was read at 734nm spectrophotometrically and IC 50 value was calculated.

FRAP assay:

To 4 test tubes ,100µg, 200µg, 300µg, 400µg and 500µg concentration of selenium nanoparticles were added and the volume in each test tube was made up to 1mL with methanol. To all the tubes, 2.5mL of 0.2M phosphate buffer(pH-6) and 2.5mL of 1% potassium ferricyanide was added and incubated at 50ºC for 20 minutes. Later, 2.5mL of 10% trichloro acetic acid was added and centrifuged at 3000rpm for 10 minutes. After centrifugation, 2.5mL of distilled water and 0.5mL of 0.1% ferric chloride solution was added. The absorbance was read at 700nm spectrophotometrically with methanol as a blank. To control and sample IC 50 value was calculated.

NO assay:

A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations sample and control (100µg, 200µg, 300µg, 400µg and 500µg) of the methanol extracts (10mg/mL) and incubated at 25°C for 180 minutes. The extract was mixed with an equal volume of freshly prepared Griess reagent (Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use). The absorbance was measured at 546 nm using a. Gallic acid was used as the positive control.

Antitumor activity:

MTT assay were done to check the Antitumor activity of the selenium nanoparticles. Single cell suspension of 10^6 cells/ml was prepared by trypsinization. 100ul of cells at concentration of 10^3- 10^6 cells/ml per well was added. For each assay, set blank wells (containing medium only), untreated control wells and test wells. Incubate at 37^0C with 5% CO_2 for 48hrs. if testing for cytotoxicity, treat cells with appropriate concentration of toxic compound for 24-48hrs. 10 µl MTT solution were added and incubate for 2to 3 hours at 37^0C until purple formazan crystals appears. Then 100 µl of DMSO was added to dissolve purple formazan crystals. Incubated for 10 mins and quantify absorbance/ optical density (OD) using a micro-titer plate reader at 570nm. The percentage of cell proliferation was calculated by following formula:

% Cell proliferation = (Average OD of test/ Average OD of control) X100

RESULTS AND DISCUSSION

Synthesis of selenium nanoparticles:

Five different mulberry leaves were collected and dried. All the five different leaves were allowed to do Soxhlet extraction and distillation process to collect the secondary metabolites for further analysis like UV spectrophotometer (as shown in figure 3).

UV- visible spectrophotometer analysis:

The UV-visible spectrophotometer in the range of wavelength from 200 to 300nm was observed for mulberry leaves extract synthesized selenium nanoparticles was and maximum at 265nm. Maximum selenium nanoparticles were synthesized from *Allium sativum* showed maximum wavelength at 260nm and 205nm [16, 15], Sowndarya et al reported a maximum wavelength of selenium nanoparticles at 420nm for *Clausena dentana* plant leaf extract [19] and for *Terminalia arjuna* leaves it was at 390nm[21].Se-NP from garlic cloves showed it maximum absorbance at 257–357 nm(34).

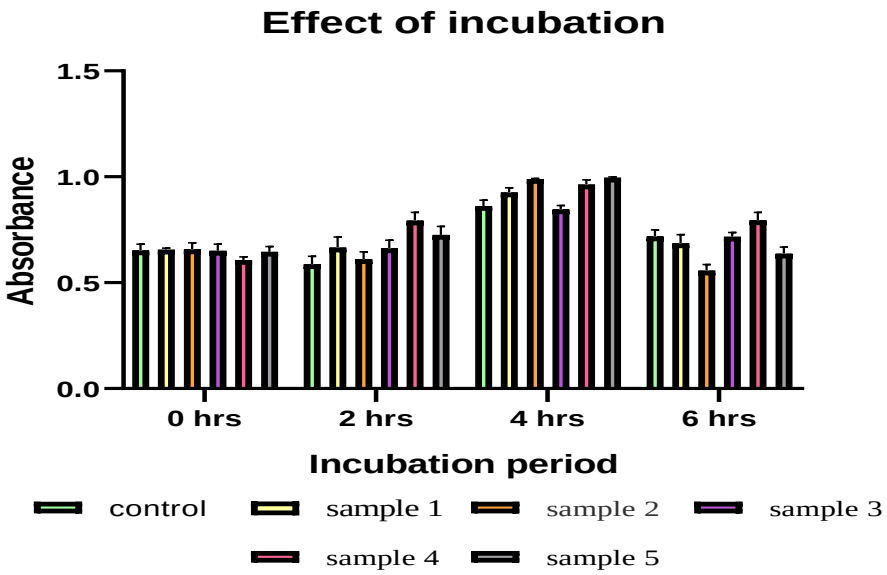

Figure 3: Absorbance of selenium nanoparticles from 5 different mulberry leaves at different time intervals.

Characterization of selenium nanoparticles

SEM analysis:

Selenium nanoparticle synthesized from mulberry has shown particle size of 397nm in SEM analysis (as shown in Figure 4). Some of the selenium nanoparticles showed particle size from a range of 200-500nm which is polygonal (1) and showed oval shape with a smooth surface and particle size of 50-150nm (17). Green-synthesized SeNP's from lemon peels exhibited particle size at 40-100 nm showed spherical, cylindrical, or rectangular in shape (34).Se-Np synthesized from *Rhizobium pusense* exhibited spherical shape and average size between 90 and 170 nm (35).

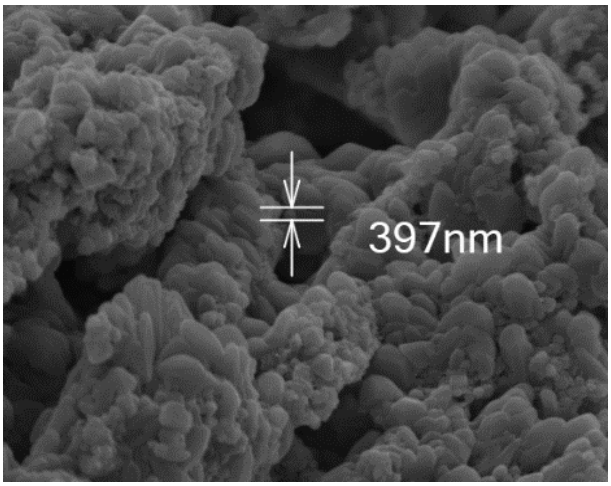

Figure 4: SEM analysis showing particle size of selenium nanoparticles.

XRD analysis:

Selenium nanoparticles synthesized from mulberry showed broad peak at 2Θ angle of 20-25^{0}which means it is crystalline in form (as shown in Figure5). Previously synthesized nanoparticles showed broad peaks at 15-35^0 which suggests that sample is not crystalline [1] and biological synthesized nanoparticles exhibited maxima peaks at 25-30^0 which determines that the sample is nanocrystalline [17] and crystallinity of selenium nanoballs showed broad peaks at lower angles confirming the amorphous/ non crystalline nature of sample [11]. Biologically synthesized Se-NP's from *bacillus megaterium* showed several peaks from 23-72°indicates the face centered cubic in structure and crystal in nature (36).

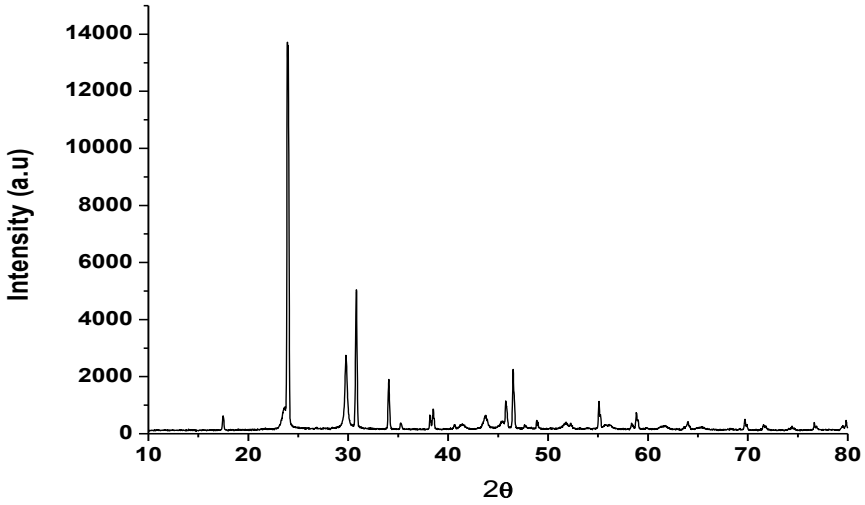

Figure 5: XRD analysis of selenium nanoparticles.

FTIR analysis:

FTIR analysis is helpful to determine which functional group is responsible for the formation of the nanoparticles. FTIR analysis showed stretched peaks at 900-1150cm $^{-1}$ indicates the presence of alcohols, alkene, amine, many stretched peaks at 1400-1750 cm $^{-1}$ indicates the presence of carbonyl group, methyl group, aldehyde group, esters, amine, nitro groups, aromatic ring, ketones, a long stretched peaks at 2750-3000 cm $^{-1}$ indicates the presence of C-H aldehyde group, alkane, carboxylic acid and a group of stretched peaks at 3500-3750 cm^{-1} indicates the presence of alcohols amines were responsible for selenium nanoparticles synthesis from mulberry leaves(figure6). Previous studies revealed that alcoholic groups and aromatic ring were responsible for the formation of selenium nanoparticles from parsley extract (14) and hydroxyl group and C-H groups were responsible for the formation of selenium nanoparticles (18). In a study, selenium nanoparticles synthesized using citrus limon fresh peels showed peak between 3200 and 3300 cm⁻¹confirmed the presence of O–H bonded stretching indicating the presence of alcohols and phenols, and other peaks at 1600–1700 cm⁻¹confirmed the presence of amide group (37).SeNP's from tea plant extract showed a peak at 3389 cm^{-1} and 2906 cm^{-1} indicating the presence of alcohols, phenols and alkanes respectively (38).

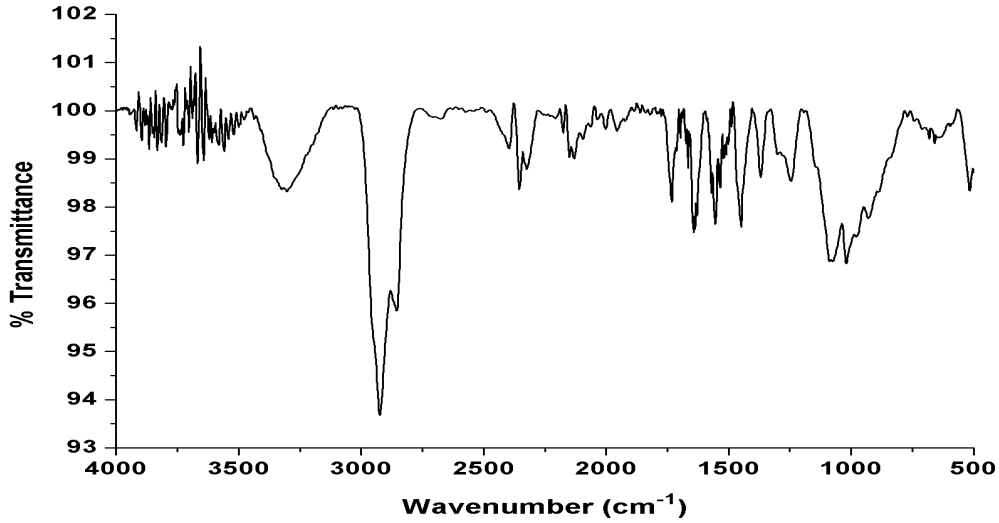

Figure 6: FTIR analysis of selenium nanoparticles.

Biological activity of selenium nanoparticles

Anti-fungal activity:

The Anti-fungal activity of selenium nanoparticles from mulberry leaves extract were examined by well diffusion method against *C. albicans* and *A. niger*. In Figures 7 and 8 selenium nanoparticles showed maximum inhibition for *A. niger* at 400µg and in *C. albicans*400µg at when compared with control (table 1). Shakibaie et al reported selenium nanoparticles synthesized from *Bacillus species* exhibited inhibition against *A. fumigatus* and *C.albicans*(39). Agrobacterium-derived Selenium nanoparticles showed inhibition zones in Sabouraud Dextrose agar media (6). Previous studies reported Se-NP from microbial source showed MIC at 0.0625mM concentration against *Rhizoctonia solani* (36). Biogenic SeNP showed 16 mm zone of inhibition at 100 µg/mL and its combination with tea plant extract showed the highest zone of 18mm against S. aureus. In screening for antifungal activity, SeNP

along with plant extract showed 14mm zone of inhibition compared to SeNP alone was 11mm at 50 µg/mL concentration against C. albicans (38).

Tabular column 1: The minimum inhibition concentration of Nanoparticles.

Selenium Nanoparticle in µg	*A.Niger*		*C.albicans*	
	Control	Sample	Control	Sample
100µg	21	16	16	15
200µg	21	21	17	19
300µg	22	25	18	19
400µg	24	29	19	20

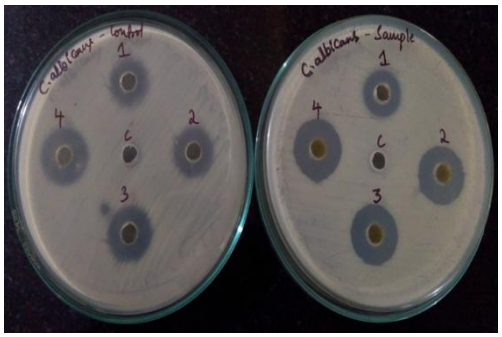

Figure 7: MIC plate against *C. albicans.*

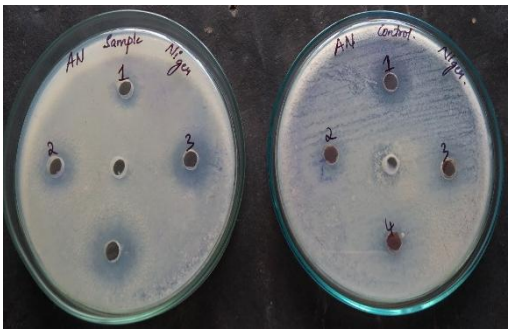

Figure 8: MIC plates against *A. niger.*

Antibacterial activity: Resazurin method:

The minimum inhibition concentration for selenium nanoparticles (sample) was maximum for *Staphylococcus aureus* (Sa) and *Streptococcus mutans* (St) was at 500µg of concentration when compared with remaining organisms. For control, the minimum inhibition concentration was maximum for *Bacillus cereus* (Bc) at 1000 µg of concentration (Figure9). Previous studies revealed that selenium nanoparticles synthesized from microbes showed Anti-bacterial activity against Staphylococcus aureus, Bacillus subtilis, E. coli and *Pseudomonas aeruginosa* by disc diffusion method (40). Selenium nanoparticles biosynthesized from propolis extract showed MIC against *staphylococcus aureus, Bacillus cereus, E. coli, Salmonella typhi, streptococcus mutans and Pseudomonas aeruginosa* resazurin microtiter plate method [41]. The MICs of synthesized Se-NPs by *Rosmarinus officinalis* extract for *S. aureus*-16 µg/mL, *S. mutans*-32 µg/mL, *E. coli*-128 µg/mL, and *P. aeruginosa*-64 µg/mL (42).

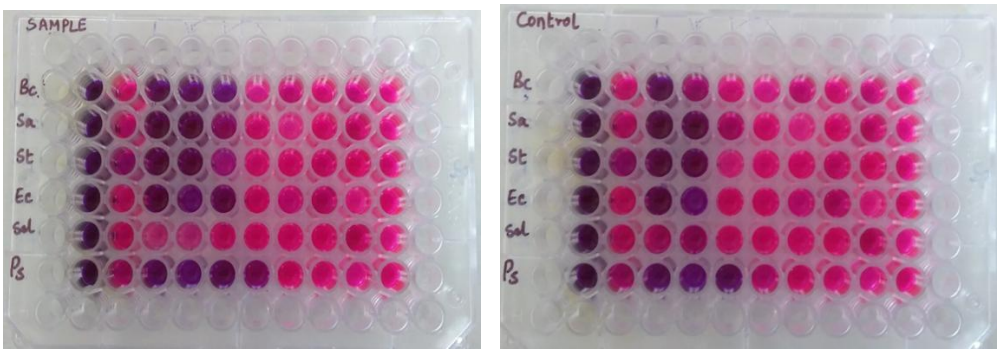

Figure 9: Resazurin method of selenium nanoparticles (sample and control).

Antioxidant activity

DPPH assay:

Antioxidants are compounds which prevent oxidation or damage cells by releasing a greater number of free radicals. The percentage of the absorbance values against different concentrations of selenium nanoparticles (control and sample) were calculated. The IC50 value of sample was more when compared with the IC50 value of control. The IC50 value of sample was 48.57µg/ml and control were 46µg/ml (figure 10). *Allium sativum* synthesized nanoparticles exhibited high antioxidant activity when compared to control (43). Selenium nanoparticles from four different tea varieties showed antioxidant activity in DPPH assay (44). Five different concentrations of nanoparticles synthesized from thymus vulgaris showed better antioxidant activity at 40µl concentration (45).

ABTS assay:

The inhibition of selenium nanoparticles was determined by calculated IC50 value of control and sample. The IC50 value of control was more than the sample. The IC50 of control was 49.3µg/ml and sample were 48.08µg/ml (figure 10). Percentage value of selenium nanoparticles stabilized by chitosan was higher in DPPH assay when compared to ABTS assay (46), selenium nanoparticles from *allium sativum* showed more percentage in ABTS assay in which rutin is the standard (43). Green synthesized nanoparticle from *Festuca arundinacea Schreb* showed maximum antioxidant activity at 4.5 mg/mL concentration (47).

FRAP assay:

In FRAP assay, IC50 of sample and control was almost similar. The IC50 value of sample were 41.069µg/ml and control were 41.54µg/ml (figure 10). Higher antioxidant activity was shown by silver nanoparticles 997.4±5.2 µmol than Rheum extract (48). In comparison with biogenic SeNP with chemically synthesized SeNP in FRAP assay. EC1 value of B-SeNP's was 155.02±0.93 µg/ ml and C-SeNP's was 178.89±1.84 µg/ ml (49). Reducing power of tea plant extract along with SeNP showed higher antioxidant activity than plant extract alone at five different concentrations (38).

Nitric oxide assay:

The percentage inhibition of selenium nanoparticles was known by calculating its IC50 value. The IC50 value of sample was more than the IC50 of control. The IC50 of sample was 49.08µg/ml and control were 43.5µg/ml (figure 10). Two concentrations of selenium nanoparticles (LC_{10} and LC_{25}) from *Penicillium chrysogenum* showed antioxidant activity of 148µmol/L and 332 µmol/L respectively compared to control of 136.75 µmol/L (50).

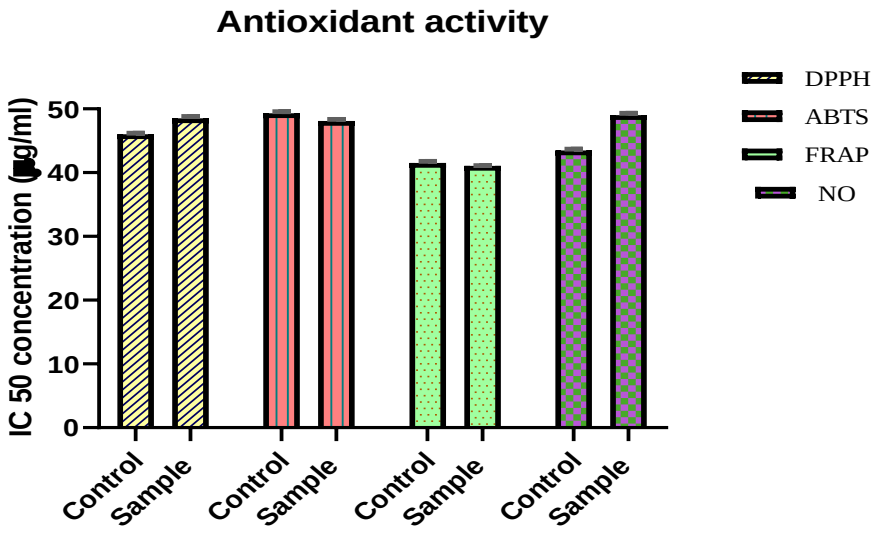

Figure 10: Four different Antioxidant activities (including DPPH, ABTS, FRAP and NO) of selenium nanoparticles (control and sample).

Antitumor activity:

Antitumor activity of selenium nanoparticles was observed against MCF-7 breast cell lines (figure 11). The percentage inhibition of selenium nanoparticles was calculated by using the formula given above.

% Of Inhibition for 10ul = 77.88%

% Of Inhibition for 25ul = 87.96%

LBP-GT-SeNP's PC 12 against H_2O_2 induced toxicity. The viability of cells incubated with H_2O_2 only was reduced to 50%, while cells pretreated with LBP-GT-SeNP's and then treated with H_2O_2 exhibited 90% viability demonstrating that SeNP were able protect H_2O_2 induced injury [18]. In a review of SeNP showed anticancer activity against many cancer cells namely colorectal, breast, liver, lung, cervical and prostate cancer cell lines (51). In cytotoxic effect of SeNP's at 100 µg/mL concentration against Caco-2 cells showed cell viability of 83.1% at 24hrs and 78.8% after 48hrs (52).

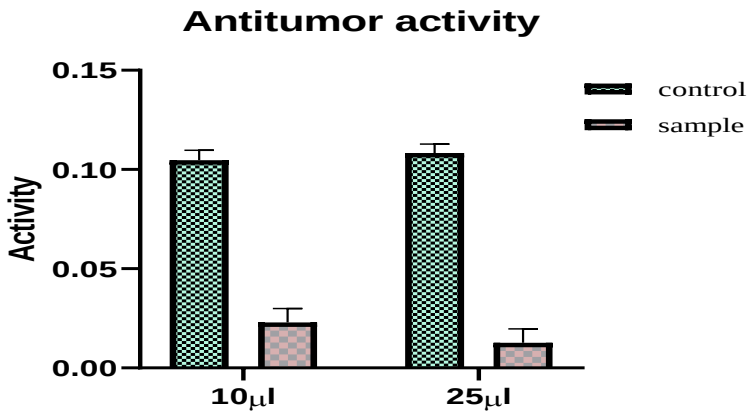

Figure 11: Antitumor activity of selenium nanoparticles (control and sample).

CONCLUSION

In conclusion, this study demonstrated that selenium nanoparticles from mulberry leaves exhibited antimicrobial including both bacterial and fungal activity by inhibiting the growth of microorganisms. These nanoparticles were effective against breast tumor cell lines by reducing the number of tumor cells under *invitro* conditions. SeNP also possessed antioxidant activity by reducing the free radicals in DPPH, ABTS, FRAP and NO assay. FTIR analysis showed presence of alcohols, carbonyl group, methyl group, aldehyde group, esters, amine, ketones, amines were responsible for the formation selenium nanoparticles from mulberry leaves. The prospective use of synthesized selenium nanoparticles in a variety of industries such as medicine, agriculture, and environmental remediation can be investigated. The toxicity and biocompatibility of SeNP's can be evaluated for their safe use in drug delivery systems.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

SeNP - Selenium nanoparticles
SEM - Scanning electron microscope
XRD - X- ray diffraction
FTIR - Fourier transform infrared
DPPH - 2,2-diphenyl-1-picryl-hydrazyl-hydrate
ABTS - 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
FRAP - Ferric ion reducing antioxidant power
NO - Nitric oxide
MTT - 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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