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# A Newly Synthesized Silver Nanoparticles Mediated Phytomedicine from *Linum usitatissimum* for Dental Caries

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The present study deals with the green synthesis of silver nanoparticles using seed extract of *Linum usitatissimum* (Flaxseed) with ethanol as solvent and examined for anticariogenic activity using disc diffusion method along with antioxidant activity using DPPH scavenging studies. The ethanolic seed extract revealed the presence of saponins, tannins, alkaloids, glycosides, counarins, anthocyanins, carbohydrates, leucoanthocyanin and xanthoproteins. Characterization of synthesized AgNPs were confirmed by FTIR to confirm the functional group involved in reduction of $Ag^{+}$ ions, SEM to know the morphology of synthesized nanoparticles and XRD to study the crystallographic structure of nanoparticles. The antioxidant activity of synthesized AgNPs showed that the maximum inhibition of 88.88% for the seed-AgNPs and compared with the standard ascorbic acid (90.74%) was observed at concentration (100 µg/ml). The anticariogenic activity of synthesized AgNPs using the seed of *Linum usitatissimum* showed the maximum zone of inhibition for *Streptococcus salivarius* with the radius of 12 mm at a higher concentration of 100 µg/ml. Therefore, it is proposed that the synthesized seed-AgNPs played the efficient role against the antioxidant activity and forms the potential source for anticariogenic activity in dental caries application.

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*Keywords: Linum usitatissimum; antioxidant activity; DPPH scavenging studies; anticariogenic activity; dental caries.*

## 1. INTRODUCTION

Over the past two decades, nanobiotechnology has been considered as the breakthrough in the fields of Medicine, Environmental biology, Textiles, Agriculture, Food Technology, and many more. Because of its distinct characterization, novel properties, and high energy consumption, it is used for both industrial and pharmaceutical purposes. Cu NPs, Ag NPs, Au NPs, $TiO_2$ NPs, ZnO NPs, CuO NPs, FeO NPs, MgO NPs, etc. [1] are some of the common nanomaterials used in nanotechnology and its wide area of application had been studied by various researchers. Amidst all silver nanoparticles is notorious for their bactericidal and fungicidal activity [2]. Project on Emerging Nanotechnologies (PEN) identified that out of 1300 nanotechnology-related products 313 products use silver nanoparticles as it makes it be the boomed NPs in consumer products application [3]. Varies have been employed for the synthesis of AgNPs, methods such as chemical, physical, biological, and photochemical methods are used. Each method has its advantages and disadvantages associated with them. Among all green synthesis of silver nanoparticles is considered as an eco-friendly and low-cost method for the synthesis of nanoparticles. AgNPs hailed the properties of anti-cancerous [4] , anticandidal [5] , antibacterial [6], anti-cariogenic [7] , antimicrobial [1] and antioxidant [8] properties. One of the common causes of tooth loss and oral pain in all age groups of people is dental caries. Even after improvements in oral health, the constancy of dental carries keeps occurring in the global population of several countries. WHO pointed out that substandard oral health may also lead to chronic diseases and affects the quality of daily life [9] . Dental caries is nothing but the damage of dental hard tissue due to bacterial fermentation. This bacterial fermentation of our daily dietary carbohydrates releases some acidic by-product which triggers the formation of dental caries and affects the oral health of a person. The bacteria belong to the genera of Actinomyces, Bifidobacterium, Eu-bacterium, Lactobacillus, Parvimonas, Rothia, Propionibacterium, Enterococcus faecalis, Scardovi and Prevotella [9] are commonly responsible for causing dental caries. Dental caries is of three types based on the location where and how they occurred, they are primary caries, secondary caries, and arrested caries. *Linum usitatissimum* (Flaxseed) which comes under the family Linaceae is one of the oldest crops which had been put into use by mankind for several purposes which include oil extraction, fibers, textiles, weaving fishnets, twines, and often used for human and animal consumption. Flaxseed is sometimes referred to as linseed which is interchangeable [10]. Flaxseed got its growing popularity as it is rich in protein content and also used as good nutritional product and traditional medicine in ancient times [11]. It is well known for its therapeutic values as it is rich in omega-3 fatty acid ALA, fiber and lignans, palmitic acid, stearic acid, oleic acid, and linoleic acid which provides various health benefits. The concentration of the acid and other contents various depending upon which part of the plant is taken for extraction. Some prominent uses of flaxseed are used to reduce blood pressure, reduce the risk of cancer, used in the treatment of kidney diseases, rheumatoid arthritis, and provides certain health benefits to people with type 2 diabetes. The flaxseed is recognized for its role in reducing total cholesterol and platelet aggregation [12]. Therefore, this study was focused on the green synthesis of AgNPs using the seeds of *Linum usitatissimum* along with determining the anti-cariogenic activity and antioxidant properties of synthesized silver nanoparticles.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Material

The seeds of *Linum usitatissimum* (Flaxseed) were collected in the month of July from Gandhi Market, Tharanallur, Tiruchirappalli District, Tamil Nadu, India.

### 2.2 Preparation of Ethanolic Extract Using *Linum usitatissimum*

The seeds *of Linum usitatissimum* were collected and dried in broad sunlight for one day. After ensuring the seeds are completely dried and it is subjected to a grinder, to make into coarse powder form. The powder was collected and kept in an air-tight container and reserved in a dark and dry place for future study. The 20g of powdered sample was mixed with a 50ml prepared ethanol solution. The extraction of *Linum usitatissimum* was achieved through the hot percolation method.

## 2.3 Optimization and Synthesis of Silver Nanoparticles

In 50ml standard conical flask 1mM $AgNO_3$ was prepared followed by addition of 2.5ml of ethanolic extract of seeds of *Linum usitatissimum* at different concentrations (25µl, 50µl, 75µl and 100µl). The mixture was subjected to constant stirring to avoid contamination so that it makes ambient condition for silver to get reduced into $Ag^+$ ions. The change in colour of the solution from white to dark brown denotes the AgNO3 formation and it was confirmed using UV-Vis spectroscopy.

## 2.4 Phytochemical Screening of *Linum usitatissimum*

The preliminary qualitative analysis is performed to find the presence of various primary metabolites in *Linum usitatissimum* extract and the quantification of phytochemicals such as flavonoids, tannins, saponins, alkaloids, phenols and terpenoids was analyzed using the standard procedure [13].

## 2.5 Characterization of Synthesized Silver Nanoparticles

Characterization of synthesized AgNPs were done to understand the functional group involved in the reduction of silver and its characteristics wavelength using FTIR and UV-Visible spectroscopy. The morphology, size, crystalline nature, and elemental composition using SEM, XRD, EDOX.

### 2.5.1 UV-Visible Analysis

The ethanolic extract of seed of *Linum usitatissimum* was mixed with silver nitrate. The change of transparent white solution to dark brown color indicates the AgNPs formation during reduction process. UV-Visible spectroscopy studies were performed to analyze the optical properties of AgNPs. The absorbance was noted between 400-500 nm.

### 2.5.2 FTIR Analysis

Fourier Transform Infrared Spectroscopy was performed to analyze the chemical properties like organic, inorganic and polymeric properties in the synthesized silver nanoparticles. FTIR absorbs spectra in the range between 400$cm^{-1}$ to 4500$cm^{-1}$. The different peaks that obtained denotes the discrete functional group involved in the silver ion reduction.

### 2.5.3 SEM Analysis

The dried synthesized silver nanoparticles without moisture content are subjected to SEM analysis in order to find the morphology of the AgNPs were taken by using JEOL-JEM 2100 operated at 20kV.

### 2.5.4 XRD Analysis

The x-ray diffraction is used to characterize the crystalline structure, grain size and nature of the crystal of silver nanoparticles.

### 2.5.5 EDX Analysis

Energy dispersive X-ray (EDX) sometimes referred to as EDAX or EDS were performed to analysis the elemental composition of the nanoparticles. The graph was obtained by taking the number of X-ray counts on Y-axis and the energy in KeV as X-axis.

## 2.6 Antioxidant Activity (DPPH Assay)

The antioxidant activity of the synthesized AgNPs of the seed extract of *Linum usitatissimum* were performed on the basis of scavenging effect on the stable DPPH free radical activity. To the 980µl of ethanol solution add 250µl of DPPH with different concentration (20-100µl/ml) of standard antioxidant drug ascorbic acid. The same process was repeated for the various concentration of synthesized silver nanoparticles followed by vigorous shaking. Absorbance was taken at 540nm using UV spectrometry. Percentage of inhibition was found out to determine the test sample's radical scavenging activities which is calculated according to the following equation:

Percent (%) inhibition of DPPH activity =
[(C- T)/ C] x 100

Where C and T are the absorbance values of the test and of the blank sample, respectively.

### 2.6.1 Collection of Test Pathogens

The anti-cariogenic activity of synthesized silver nanoparticles from flaxseed was exhibited against *Streptococcus mutans* (MTCC 890), *Streptococcus salivarius* (MTCC 13429), *Streptococcus sobrinus* (MTCC 33479) and *Staphylococcus aureus (*MTCC 25923) were

prepared as test organisms. All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India.

### 2.7 Determination of Anti-cariogenic Activity by Disc Diffusion Method

The disc diffusion method is used to determine the anti-cariogenic activity of the synthesized seed-AgNPs of *Linum usitatissimum*. The obtained nanoparticles are allowed to bind with the paper disc for sometimes. 25 ml of Mueller-Hilton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organism followed by various concentrations of isolated compounds of 60,80 and 100 mg/ml. Filter paper disc loaded with 10µl of amoxicillin was used as positive control. Negative control was prepared using ethanol as solvent. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was recorded in millimeter.

#### 2.7.1 Determination of MIC and MBC

To the 2 ml of Nutrient Broth (NB) add 50 µl of various culture in each test tubes respectively which is followed by the addition of discrete concentration of the isolated nanoparticles (10,20,40,60,80 and 100µg/ml). The procedure was repeated on the test organisms using the standard amoxicillin. A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then inoculated at 37 ºC for 24 hours. After incubation the tubes were then examined for turbidity of microbial growth. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which does not show any growth and inoculated on sterile nutrient agar by streaking. Nutrient agar only was streaked with the test organisms, respectively, to serve as control. Plates were then inoculated with bacteria and then incubated at room temperature at 37ºC for 24 hours. After incubation, the lowest concentration at which no visible growth was noted as the minimum bacterial concentration.

## 3. RESULTS AND DISCUSSION

### 3.1 Qualitative Analysis of Synthesized Silver Nanoparticles from *Linum usitatissimum*

The phytochemical screening of *Linum usitatissimum* seed extract using different tests standards was represents in (Table 1) revealed that the presence of terpenoids, flavonoids, saponin, tannin, alkaloids, steroids, glycosides, phlobatannins, counarins, emodins, anthraquinone, anthocyanins, carbohydrates, leucoanthocyanins, cardiac glycoside, xanthoproteins, phenols which are known to possess physiological and medicinal activities while, proteins were absent. Qualitative analysis of seed of *Linum usitatissimum* reveals the presence of phytochemical constituents which was primary responsible for their biological activity. Previously [14] reported the presence of phenolic and flavonoid compounds from the flaxseed sprouts. The present result is in accordance with [15] which shows the presence of Steroids, Terpenoids, Tannins, Saponins, Anthocyanins, Emodins, Glycosides, Alkaloids, Phenols, Flavonoids from the seed extract of *Linum usitatissimum*. Another phytochemical screening study conducted on *Linum usitatissimum* revealed the presence of tannins, flavonoids, terpenoids, phenols, proteins and amino acids [16].

### 3.2 Quantitative analysis of Synthesized Silver Nanoparticles from *Linum usitatissimum*

From the qualitative analysis, the phytoconstitutents present in *Linum usitatissimum* seed extract in different amount have been reported. The phytoconstiuents with higher quantity present is saponins followed by flavonoids, alkaloids, tannins, phenols and terpenoids. The compositions of phytoconstituents are flavonoids (0.018mg/g), alkaloids (0.012 mg/g), tannin (0.007 mg/g), saponins (0.026 mg/g), phenol (0.005 mg/g) and terpenoids (0.004 mg/g).

### 3.3 Observation of Visual Color Change and UV-Visible Spectroscopy

UV-Vis spectroscopy was performed in order to confirm the presence of silver nanoparticles which was observed at room temperature. The appearance of the sample from colourless to dark brown colour indicates the bio reduction of Ag+ in ethanol extract. At 437nm the intense bands at the UV visible absorption spectrum of the seed extract of *Linum usitatissimum* was noted (Fig. 1). The previous study proposed the maximum absorption of silver nanoparticles occurred at the range of 385-400nm in UV-Vis absorption spectroscopy of the hydroalcoholic

extract of *Linum usitatissimum* [17]. The study conducted on *Indigofera aspalathoides* also showed the maximum absorption of silver nanoparticles occurred at the peak of 420 nm [18].

### 3.4 Identification of Functional Group Using FTIR Spectroscopy

FT-IR spectroscopy is used to find the functional groups involved in the reduction of silver ions in our sample. The FT-IR spectra of synthesized silver nanoparticles from seed extract of *Linum usitatissimum* is given in the (Fig. 2) and the functional group which play role in the reduction of $Ag^{+}$ ion is listed in the (Table 2). The present result is in accordance with the [19] that indicates the occurrence of strong peak at 3438.11cm-$^{1}$ and medium peak at 670.15cm-1 which reveals the presence of primary amines and halo compounds respectively. The non-occurrence of peak at range 1543cm-1 denotes the absence of protein in the sample which is also coincide with the results of [17]. The FTIR analysis of synthesized AgNPs from *Jatropha gossypifolia* showed the maximum IR bands at 3480, 3342, 3231, 2115 cm-1 and minor bands at 1635,685,655cm$^{-1}$ [20].

**Table 1. List of phytochemical constituents present in ethanolic seed extract of *Linum usitatissimum***

| S.NO | Metabolites | Present/Absent | Concentration |
|---|---|---|---|
| 1. | Terpenoids | Present | ++ |
| 2. | Flavonoids | Present | +++ |
| 3. | Saponins | Present | +++ |
| 4. | Tannins | Present | +++ |
| 5. | Alkaloids | Present | +++ |
| 6. | Steroids | Present | ++ |
| 7. | Glycosides | Present | ++ |
| 8. | Phlobatannins | Present | + |
| 9. | Proteins | Absent | - |
| 10. | Counarins | Present | +++ |
| 11. | Emodin's | Present | + |
| 12. | Anthro quinones | Present | ++ |
| 13. | Anthocyanins | Present | +++ |
| 14. | Carbohydrates | Present | +++ |
| 15. | Leuco anthocyanin | Present | +++ |
| 16. | Cardiac glycosides | Present | ++ |
| 17. | Xanthoproteins | Present | +++ |
| 18. | Phenols | Present | + |

*Note: ("+" indicates the low concentration; "++" indicates medium concentration; "+++" indicates high concentration of phytochemical constituents; "-" indicates the absence of specific phytochemical constituents)*

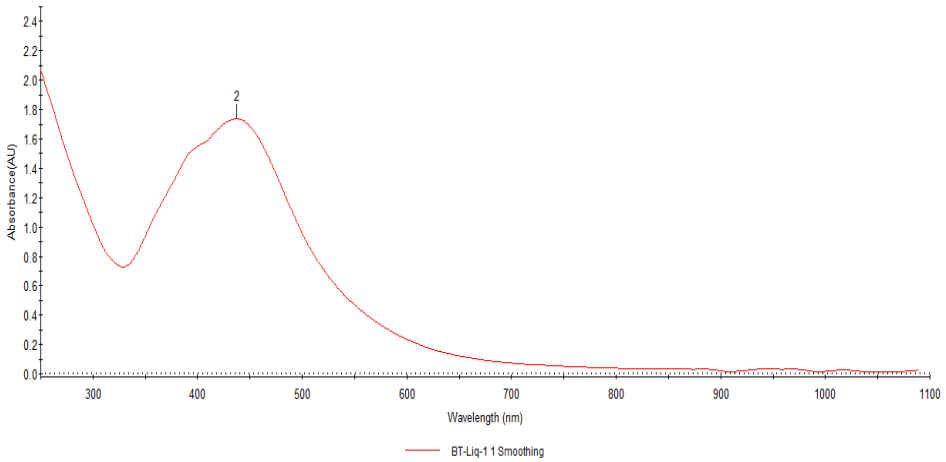



**Fig. 1. UV-Vis spectra of synthesized silver nanoparticles from seed extract of *Linum usitatissimum***

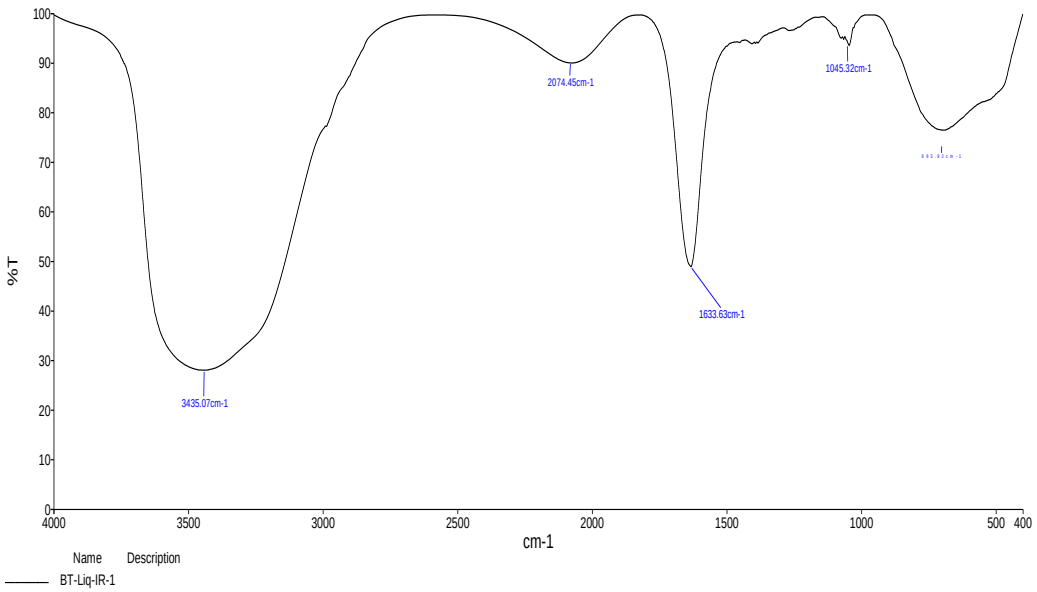



**Fig. 2. FT-IR spectra of synthesized silver nanoparticles**

**Table 2. FT-IR spectra of synthesized silver nanoparticles from *Linum usitatissimum***

| Functional group | Band | Frequency.cm-$^{1}$ |
|---|---|---|
| Primary amine | Strong band | 3435.07cm-1 resemble to O-H stretching alcohol |
| Isothiocyanate | Strong band | 2085.17 cm-1 corresponds to N=C=S stretching vibrations |
| Lipids & Amino acids | Strong band | 1633.63cm-1 resemble to ester carbonyl C=O stretching |
| Halo compounds | Medium band | 695.92cm$^{-1}$ resemble to C-Br stretching vibration |
| Carbohydrates | Medium band | 1045.32cm-1 resemble to Coupled C-C & C-O vibration |

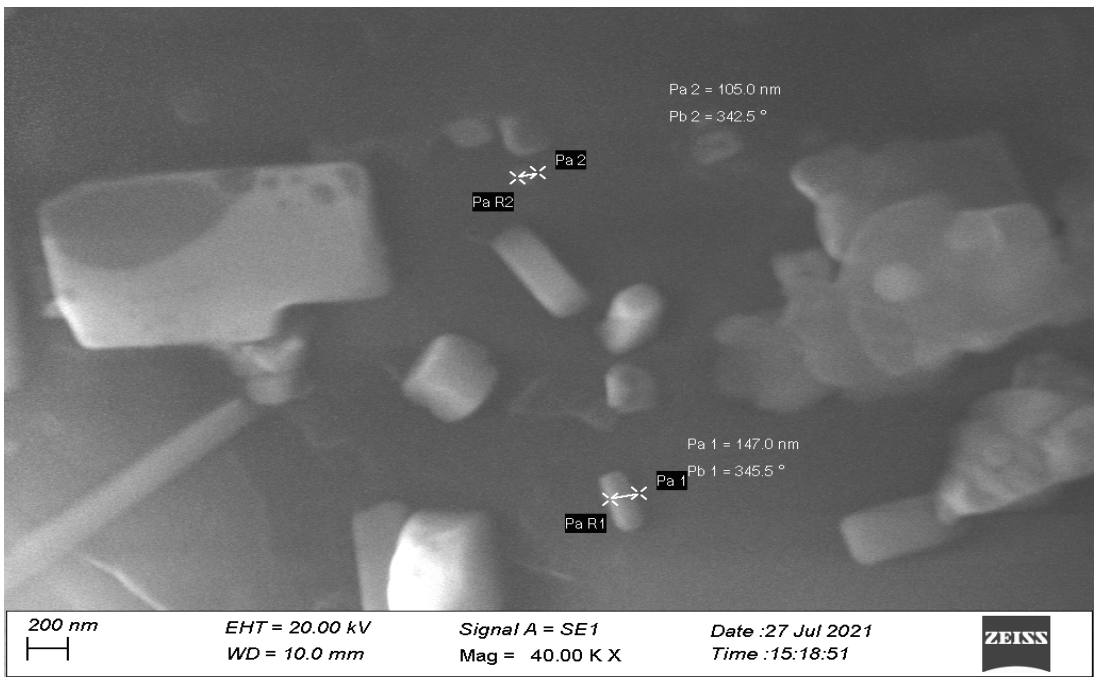



**Fig. 3. SEM image of synthesized AgNPs from ethanolic seed extract of *Linum usitatissimum***

### 3.5 SEM Image

The surface morphology of the nanoparticles has been found using the SEM image. The synthesized silver nanoparticles from *Linum usitatissimum* range from 105.0 to 147.0 nm. The SEM image reveals that the AgNPs are spherical in shape which was summarized in the (Fig. 3). The previous study by [19] also stated the presence of spherical and cubic silver nanoparticles on the surface in SEM analysis of the leaves and bark of *Pterocarpus indicus willd*. Previous studies indicated the presence of bead like spherical silver nanoparticles in the size ranges from 49-54nm in *Linum usitatissimum* [21].

### 3.6 X-Ray Diffraction

X-ray crystallography has been used to find the crystalline structure of the synthesized silver nanoparticles. The acute peaks were obtained at 2θ values 38.2586, 45.5689, 67.0203, 78.2468. The facets for the following values are (111), (222), (220), (311) respectively. XRD pattern of synthesized AgNPs was given in (Fig. 4) The prominent peaks may represent the capping agent which involved in stabilizing the AgNPs. The resulted pattern reveals the face centered cubic system of silver according to (JCPDS, File No. 04-0783). Previous study on the *Cochlospermum religiosum* revealed the presence of face centered cubic structure of silver nanoparticles which were observed at peak of 2θ values at 38.28° and 48.04° corresponding to (111) and (200) [22].

### 3.7 EDAX Analysis

Energy dispersive X-ray (EDX) spectrometer analysis was performed to confirm the elemental signal of AgNPs. EDAX spectra for synthesized silver nanoparticles from seed extract of *Linum usitatissimum* was shown in (Fig. 5) with supplementary peak of Ni, Na, Si and Cl as these represents the biomolecules attached along with the silver nanoparticles surfaces. The weight of the silver in AgNPs reduced by the seed extract of *Linum usitatissimum* was found to be 56.42%. The weight of silver in the silver nanoparticles reduced by *Terminalia chebula* bark was found to be 65.81% [23] which is close to the present result obtained. The present result is also in accordance with the study conducted on the plant *Artemisia nilagirica* where the presence of silver nanoparticles had been observed and showed the strong energy signal in the range between 2-3keV [24].

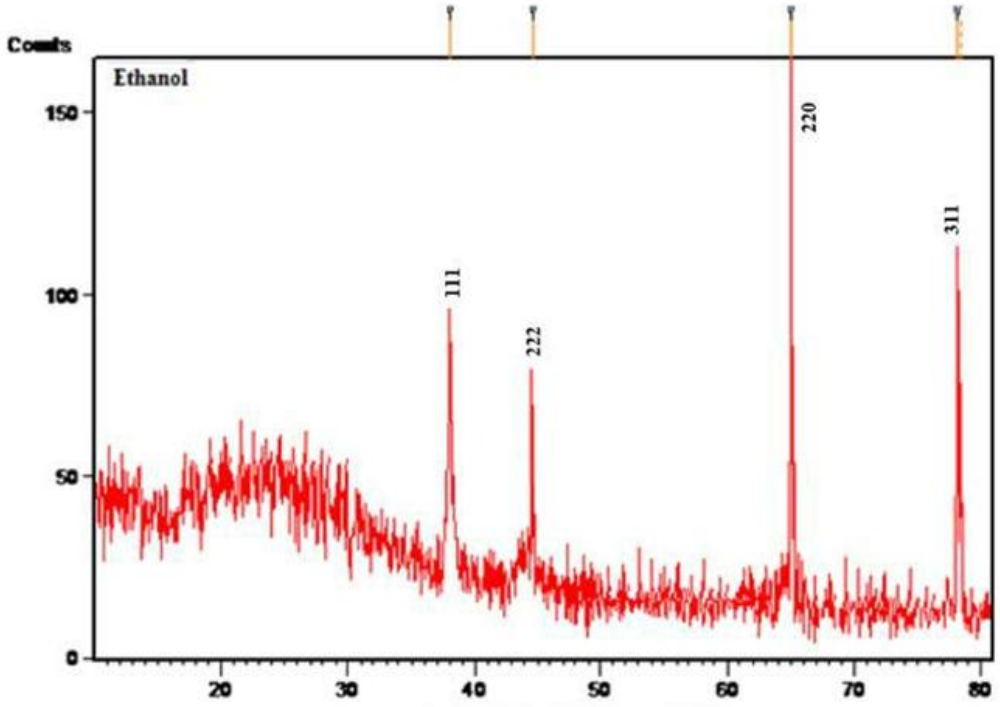



**Fig. 4. X-ray diffraction pattern of the synthesized AgNPs**

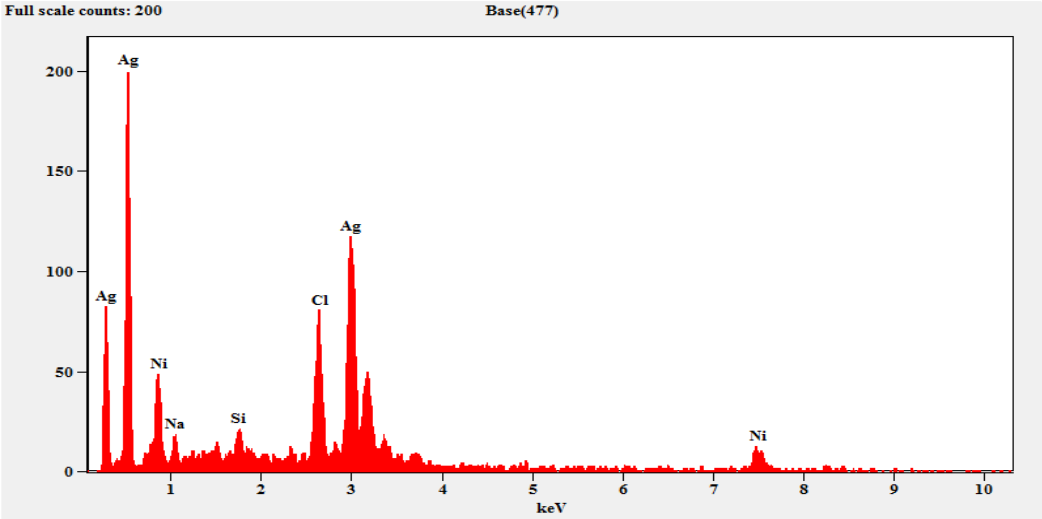



**Fig. 5. EDAX spectra of synthesized AgNPs**

### 3.8 Antioxidant Activity of Synthesized AgNPs from Seed Extract of *Linum usitatissimum* by DPPH Method

The result depicts that the silver nanoparticles synthesized using *Linum ustitalissimum* seed extract showed maximum potent of antioxidant activities at high concentration as compared with ascorbic acid. The synthesized silver nanoparticles showed scavenging effect of 88.88% at concentration 100µg/ml while ascorbic acid gave 90.74% at the same concentration which shown in (Fig. 6). The previous study conducted by [25] stated that scavenging effect of AgNPs from *Atrocarpus altilis* leaf extract is 79.79%. The scavenging effect of synthesized silver nanoparticles from *Elephantopus scaber* leaf extract showed 85.90% at 250µg/ml and 86.0% at 140µg/ml of ascorbic acid [26]. Previously scavenging activity of butanol and methanol extracts of linseed were found to be 96.2% and 93.1% respectively [27]. The result obtained from the study conducted on the *Rosmarinus officinalis* also in accordance with the present result where the antioxidant activity of silver nanoparticles was observed to be 93.5% in aqueous extract [28].

### 3.9 Anticariogenic Activity of Synthesized AgNPs from Seed Extract of *Linum usitatissimum*

The substandard quality of oral cavity health leads to chronic diseases in humans. The species which are prone to oral health includes *Streptococcus mutans, Streptococcus salivarius, Streptococcus sobrinus* and *Staphylococcus aureus*. The anticariogenic activity of synthesized AgNPs against these bacteria was investigated and results were tabulated in the (Table 3).

The synthesized silver nanoparticle shows the maximum inhibition against the *Streptococcus salivarius* at concentration of 100µg/ml. The previous study by [29] suggested the maximum inhibition against *Lactobacillus casei* by the biosynthesized silver nanoparticles from *Salmonella typhirium*.

### 3.10 Minimum Inhibitory Concentration (MIC) & Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration [MIC] for the synthesized silver nanoparticles for *Linum usitatissimum* using ethanolic extract was found followed by minimum bactericidal concentration which is presented in the (Table 4).

From the Table 4 it is understood that seed extract of *Linum usitatissimum* shows discrete anticariogenic activity against the four bacterial strains that had been tested. It has been noted that the extract is very effective against the strain *Streptococcus salivarius* at lower concentration itself followed by *Staphylococcus aureus, Streptococcus mutans* and *Streptococcus sobrinus*. So, it was confirmed that the synthesized nanoparticles from the seed extract of *Linum usitatissimum* using the ethanol extract has the efficient anticariogenic activity. The previous study by [17] showed that the

synthesized AgNPs from the hydroalcoholic extract of Flaxseed shows good anticariogenic activity against the *Staphylococcus aureus*. The present result also supported the previous study conducted on testing the anticariogenic activity of the AgNPs synthesized from *Lysiloma acapulcensis* where it shows good antimicrobial activity against *Staphylococcus aureus* [30].

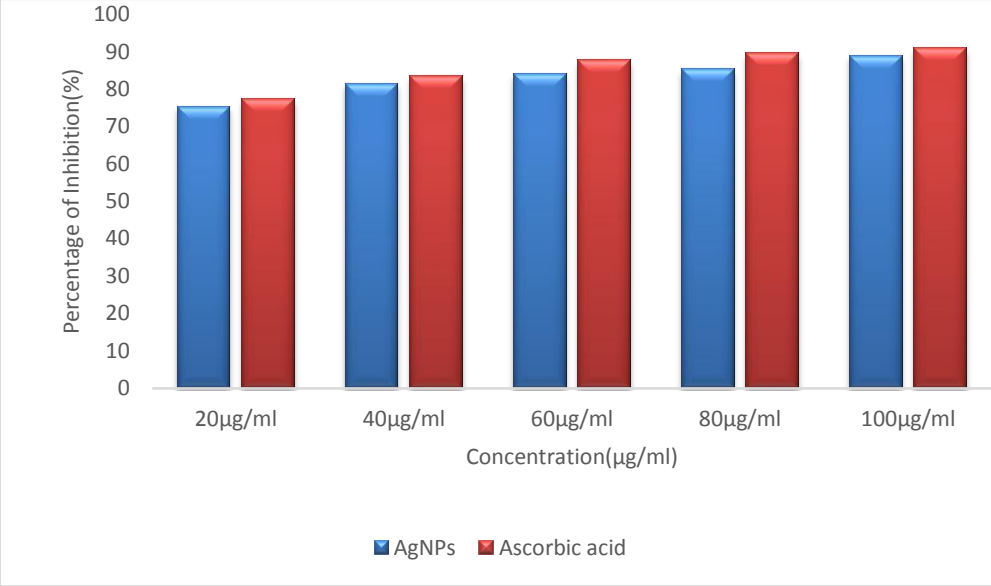



**Fig. 6. Antioxidant activity of synthesized silver nanoparticles using *Linum usitatissimum* seed by DPPH activity**

**Table 3. Anticariogenic activity of Synthesized AgNPs from *Linum usitatissimum***

| Samples | Concentration (µg/ml) | Organisms/ Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | Isolated Organisms | | | |
| | | *Streptococcus mutans* | *Streptococcus salivarius* | *Streptococcus sobrinus* | *Staphylococcus aureus* |
| Synthesized AgNPs | 60 | 0 | 0 | 0 | 0 |
| | 80 | 7 | 10 | 6 | 5 |
| | 100 | 9 | 12 | 8 | 7 |
| Control (Ethanol) | 10 | 0 | 0 | 0 | 0 |
| Standard (Amoxicillin) | 10 | 11 | 12 | 11 | 12 |

**Table 4. MIC & MBC of synthesized silver nanoparticles from ethanolic extract of *Linum usitatissimum***

| S.No | Organisms | Synthesized AgNPs | | Amoxicillin | |
|---|---|---|---|---|---|
| | | MIC(µg/ml) | MBC(µg/ml) | MIC(µg/ml) | MBC(µg/ml) |
| 1 | *Streptococcus mutans* | 40 | 40 | 40 | 20 |
| 2 | *Streptococcus salivarius* | 40 | 20 | 20 | 10 |
| 3 | *Streptococcus sobrinus* | 60 | 40 | 40 | 20 |
| 4 | *Staphylococcus aureus* | 40 | 20 | 20 | 20 |

## 4. CONCLUSION

The silver nanoparticles have been successfully synthesized from the seed extract of *Linum usitatissimum* using ethanol as an extract and the formation of AgNPs confirmed by UV-Vis spectroscopy. Further, the SEM image revealed that the obtained silver nanoparticles are spherical in shape and size ranges from 105.0 to 147.0 nm. Through DPPH assay it is confirmed that the synthesized AgNPs shows good inhibition percentage of 88.88% at concentration of 100µg/ml. Anticariogenic activity of the AgNPs also tested through disc diffusion method which exhibited against S. mutans, S. *salivarius, S. sobrinus* and *S. aureus*. The results shows that seed-AgNPs is more efficient against *S. salivarius* with inhibition zone of 12mm radius. Thus, silver nanoparticles obtained from the seed of *Linum usitatissimum* has been praised as an effectual anticariogenic agent in order to treat the oral cavities.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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