ANTIBACTERIAL EFFECT OF $NiSe_2$ NANOPARTICLES USING CITRUS LIMON LEAVES BY GREEN METHOD

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ABSTRACT

Recently, nanotechnology has emerged as a promising interdisciplinary section dealing with research and development in various fields. The advancements of nanotechnology-based approaches in various commercial segments and their direct effect on human life justify the need for their Ecofriendly ways of synthesis .Nanoparticles are generally described as small particles that measures Around 1 nm to 100 nm in size. The variations in shape, size, surface to volume ratio and Composition of nanosized metal nanoparticle provides them unique physical, chemical, and Biological properties that can be used in medicines, electronics, household devices, agriculture, Cosmetics, and pharmaceutical areas.

Pure $NiSe_2$ Nanoparticles were prepared by green synthesis method. The formed samples were characterized by powder XRD, EDX, SEM and Antimicrobial studies.

XRD can be used to identify single crystals, and to reveal the structure of single crystals.. SEM image shows the surface morphology of pure and $NiSe_2$ nanoparticle. From EDX is an analytical technique used for the elemental analysis or chemical characterization of a sample presence of Ni and Se were confirmed.

Antibacterial activity of $NiSe_2$ nanoparticles was performed by Agar well diffusion method against E.Coli, Pseudomonas aeruginosa, Streptococcus oralis, Staphylococcus aureus and propionibacterium acnes was purchased from MTCC, Chandihar, India. The highest zone of inhibition of $NiSe_2$ nanoparticles synthesized by Citrus Limon extract was found against Streptococcus oralis.This field is used for medicine, due to their high antibacterial activity. The

antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control.

Anti-fungal activity of $NiSe_2$ nanoparticles was performed by Agar well diffusion method against Aspergillus Niger, Aspergillus fumigates and Aspergillus flavus. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control.

Key words: green synthesis, nanoparticles, XRD, , SEM, Anti-Microbial studies, antifungal studies.

INTRODUCTION

Nanomedicine is the medical application of nanotechnology. Nanomedicine ranges from the medical applications of nanomaterial biological devices, to nanoelectronic biosensors, and even possible future applications of molecular nanotechnology such as biological machines. Current problems for nanomedicine involve understanding the issues related to toxicity and environmental effect of nanoscale materials (materials whose structure is on the scale of nanometers, i.e., billionths of a meter). Functionalities can be added to nanomaterials by interfacing them with biological molecules and structure; therefore, nanomaterials can be useful for both in vivo and in vitro bio medical research and applications.

The far, the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. Nanomedicine seeks to deliver a valuable set research tools and clinically useful devices in the near future. The National Nanotechnology Initiative expects new commercial applications in the pharmaceutical industry that may include advanced drug delivery systems, new therapies, and in vivo imaging

In recent year, the number of infections associated with antibiotic-resistant bacteria has increased. The center for Disease Control and Prevention reports that the number of annual Multidrug-Resistant Staphylococcus Aurous (MRSA) infections increased from 127,000 to 278,000 between the years 1999 and 2005. Similarly, the number of annual MRSA-related deaths increased from 11,000 to 17,000 over the same time frame. While a decline in the prevalence of MRSA infections was reported from 2005 to 2008 due to the implementation of

preventative measures, these infections remain a concern. The rise of MRSA is attributed primarily to the overuse and improper use of antibiotics. Bacteria, with their large populations and fast reproduction time, are able to rapidly develop mechanisms of antibiotic resistance when a subset of the bacteria population survives antibiotic exposure.

Antibiotic resistance may develop via multiple mechanisms. Briefly, the primary mechanisms include alteration or inactivation of the antibiotic by the bacteria, alteration of the target site of the antibiotic, alteration of a metabolic pathway to avoid the disruptive effect of the antibiotic, and reduced accumulation of the drug by minimizing its entry or maximizing clearance from the cell. To illustrate one such mechanism, inactivation of the antibiotic, antibiotic resistance may be the ability of the bacteria to adapt production of beta-lactamase enzymes which cleave the beta-lactam ring, neutralizing beta-lactam antibiotics such as penicillin. Bacteria, such as S. aurous, may use this mechanism alone, or in conjunction with other resistance mechanisms, to dramatically reduce its susceptibility to the bactericidal effects of large classes of traditional antibiotics.

For this reason, entirely new approaches to antibiotic development are necessary to keep up with the constantly changing antibiotic resistance of bacteria. Nanoparticles, which rely on entirely different mechanisms of antibacterial activity than traditional antibiotics, provide a compelling alternative. Determining the effectiveness of a nanoparticle as an antibacterial agent requires experimental techniques that measure bacteria viability after exposure. While numerous techniques have been developed to determine the antibacterial activity of nanoparticles, many of them are flawed in their own way. As a result, multiple techniques are often used in a single study to compare and confirm antibacterial results

Nickel Selenide Nanoparticles

Nickel selenide is the inorganic compound with the formula NiSe. As for many metal chalcogenides, the phase diagram for nickel (II) selenide is complicated. Two other selenides of nickel are known, $NiSe_2$ with a pyrite structure, and Ni2Se3. Additionally, NiSe is usually nonstoichiometric and is often described with the formula Ni1−xSe, with $0 < x < 0.15$. This material is a semiconducting solid, and can be obtained as in the form of a black fine powder, or

silver if obtained in the form of larger crystals. Nickel (II) selenide is insoluble in all solvents, but can be degraded by strongly oxidizing acids.

Table.3.1 Properties of Nickel Selenide nanoparticle

Element name	**Atomic mass number**	**Melting point**	**Boiling point**	**Density**	**Covalent Radius**	**Vander Waals Radius**
Nickel(Ni)	58.69	1728 K	3003 K	8.908g/cm^3	124±4pm	163pm
Selenide(Se_2)	78.96	494 K	958 K	Gray:4.81g/cm^3 Alpha:4.39g/cm^3 Vitreous:4.28g/cm^3	120±4pm	190pm

Synthesis of Nickel Selenide nanoparticles

Citrus Limon leaves is obtained around the campus of St. Xavier's college, palayamkottai. 100grms of citrus lemon leaves is measured, and cut the leaves are put into 100ml distilled water. Nickel chloride and Selenium powder were purchased from Himeda chemical reagent.50ml distilled water is taken and mixing with Nickel Chloride and Selenium powder for magnetic starring. The leaf extract is filtered (100ml) and mixing with chemicals for magnetic starring (3hours). The colour change into black with the mixture indicates the presence of $NiSe_2$ nanoparticles. The solution was taken into auto clave for 24 hours. The mixture was filtered using whatmann filter paper and the precipitate was obtained was cleaned twice by distilled water and then kept in oven under 500^0C. Finally we obtained $NiSe_2$ nanoparticles. $NiSe_2$ nanoparticles is calcinations around 250^0c .

Powder XRD diffraction

X-ray diffraction is obviously the most common tool to study the crystal structure of nanoparticles. The grain size and structure of the nanoparticles are investigated with a powder diffractometer with radiation at a diffraction angle (θ) between 20^0to80^0 ranges.

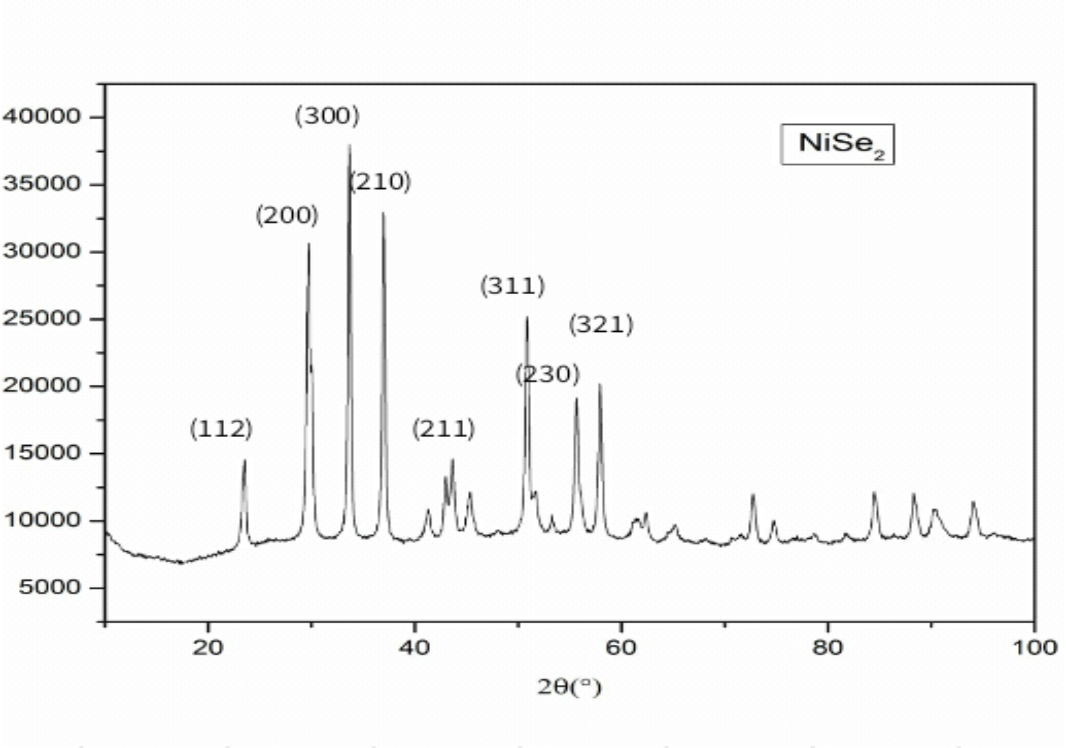

Fig.1 XRD pattern of $NiSe_2$ nanoparticles

From the powder X-ray diffraction studies, find finely sharp peaks are observed, which indicates the crystalline perfection of $NiSe_2$ nanoparticles. For the $NiSe_2$ nanoparticles the peaks observed at 23.48, 29.67, 33.67, 36.98, 50.86, 55.65, and 57.95. The XRD pattern well matches with the JCPDS FILE NO: 41-1495. Which will match the (112), (200), (300), (210), (211), (311), (230), and (321). The synthesized nanocomposite has a cubic structure with lattice parameter a= 5.991.

XRD can be utilized to evaluate peak broadening with crystallite size and lattice strain due to dislocation. The average crystallite size of the $NiSe_2$ nanoparticles is around **21.67nm.** The dislocation density (δ) is around **0.0021295** of the prepared nanoparticles. It has been reported that crystallite size below 50nm are useful in obtaining the suitable signal to noise ratio.

The surface morphology of the samples was investigated by field emission scanning electron microscopy and it's used for morphological characterization at the nanometer to micrometer scale. Fig.4.4 shows the SEM analysis of the $NiSe_2$ nanomaterial prepared using citrus lemon

leaf extract and this figure indicates that the particles are agglomeration shape. Scale 4μm and WD=10nm

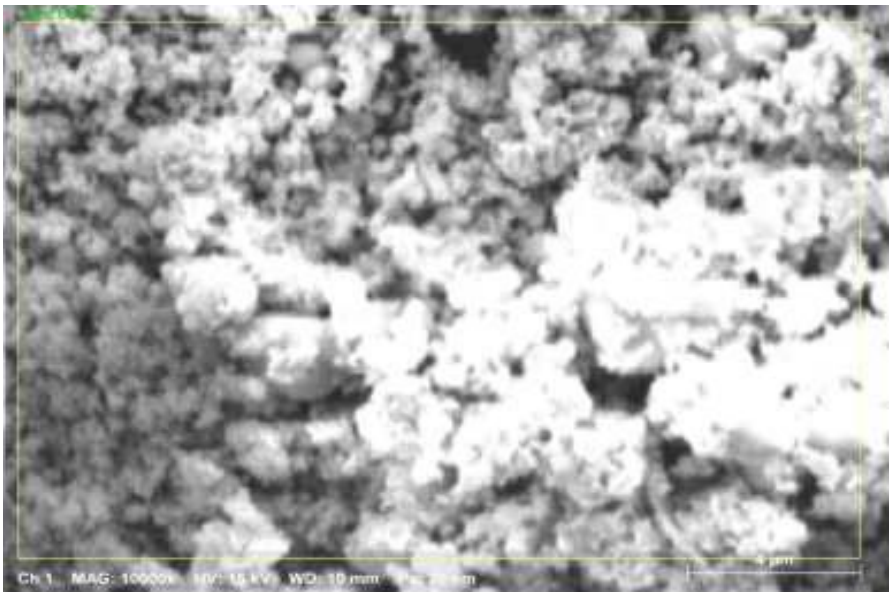

Fig. 2. SEM analysis

EDX Analysis

Energy dispersive x-ray spectroscopy is an analytical technique used for the chemical analysis or chemical characterization of a sample. It relies on an interaction of same source of x-ray excitation and a sample. The purity and composition of the prepared particles were analyzed by EDX.

Fig.4.5 shows the EDX spectrum of $NiSe_2$ nanoparticles from plant extract method. Table 4.1 shows the chemical constituents present in my sample. The table which shows the percentage of elements presents in my sample which indicates the prepared nanoparticles contains Ni and Se ions for $NiSe_2$ nanoparticles.

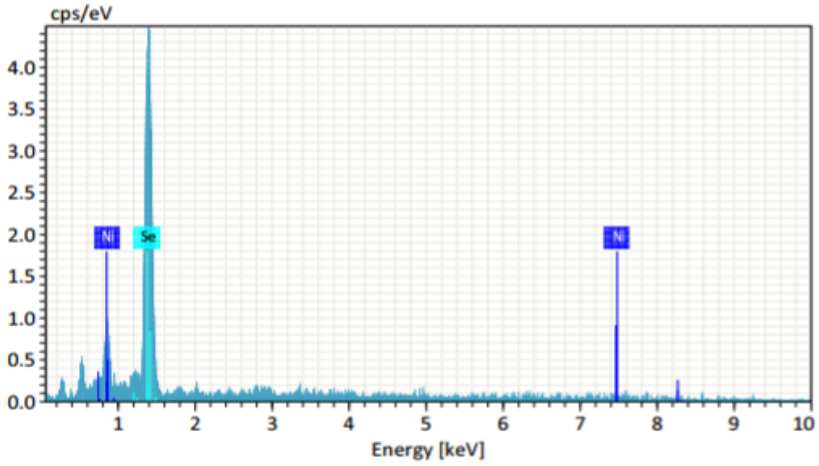

Fig.3 EDX analysis of $NiSe_2$ nanoparticles

Table :1 EDX data for $NiSe_2$ nanoparticles

Element	Atom. No	Mass(%)	Mass Norm (%)	Atom (%)	Abs. Error (%)	rel.error (%)
Se	34	58.02	90.56	87.70	3.00	5.17
Ni	28	6.05	9.44	12.30	0.60	9.94
Total		**64.07**	**100.00**	**100.00**		

Antimicrobial analysis

Antimicrobial analysis of nickel selenide nanoparticle synthesis by using Citrus Limon was done by using agar well diffusion method. An antibacterial study was done by five bacteria such as, E.Coli, Pseudomonas aeruginosa, Streptococcus oralis, Staphylococcus aureus, Propionibacterium acnes and antifungal studies were done by three fungal such as, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger.

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extract. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoclum over the entire agar surface.

Antibacterial analysis

The shelf life of a product can be extended either by adding ar5tificial preservatives or by taking hygienic measures during the manufacturing process. As the consumer trend today is towards preservative free foods with a long shelf-life, industry is being forced to rethink its manufacturing methods. Instead of adding preservative agents, increased hygienic precautions can be taken during production. A clean and hygienic manufacturing environment is an essential prerequisite in order to keep contamination related reject rates low. The utilization of surfaces in the manufacturing environment with antibacterial properties can significantly reduce contamination risks.

Pseudomonas aeruginosa

Antibacterial analysis done by using Pseudomonas aeruginosa bacteria fig 4 shows the photograph of the effect of the sample against these bacteria.

Fig: 4 Effect of sample AS1 against Pseudomonas aeruginosa.

Bar diagram 5 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. The control value **AB= 10.5±1.5**. The control of AB is used for **Gentamicin antibiotic.**

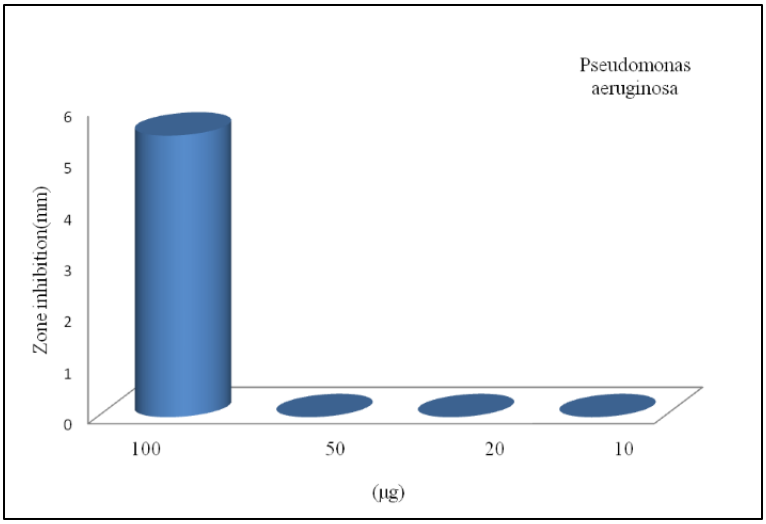

Fig.5 Bar graph of pseudomonas areuginosa (100, 50, 20, 10) µg/ml

Streptococcus oralis

Antibacterial analysis done by using Streptococcus oralis bacteria fig 6 shows the photograph of the effect of the sample against these bacteria.

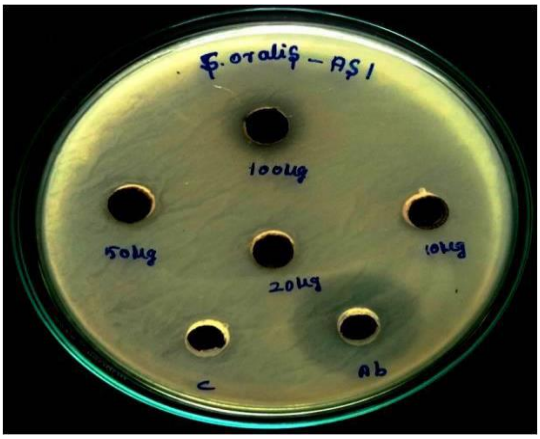

Fig: 6 Effect of sample AS1 against Streptococcus oralis

Bar diagram 7 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. The control value **AB= 11±1.0.** The control of AB is used for **Gentamicin antibiotic.**

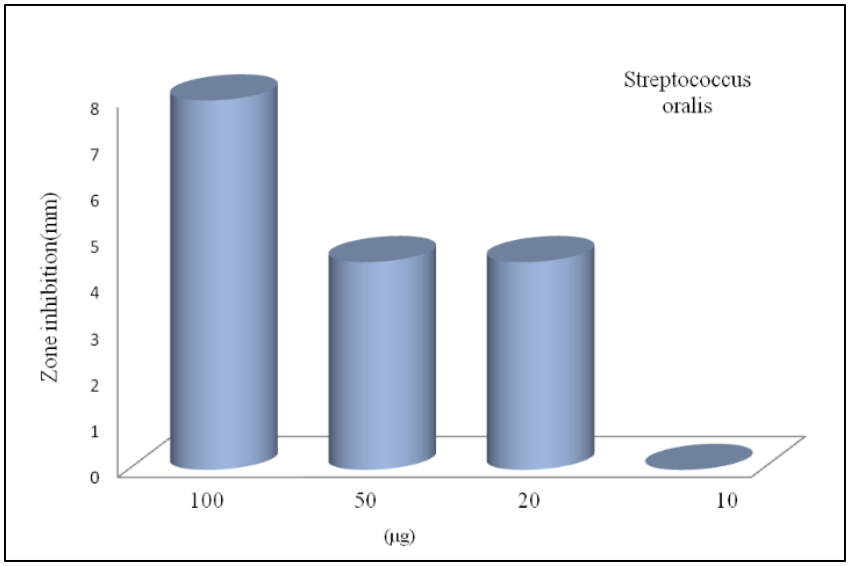

Fig: 7 bar graph of Streptococcus oralis (100, 50, 20, 10) µg/ml

Staphylococcus aureus

Antibacterial analysis done by using Staphylococcus aureus bacteria fig 8 shows the photograph of the effect of the sample against these bacteria.

Fig.8 Effect of sample AS1 Staphylococcus aureus

Bar diagram 9 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. The control value **AB= 10.5±2.5.** The control of AB is used for Gentamicin antibiotic. The zone of inhibition value increases with increase in concentration. The value is more for higher concentration i.e., 100µg/ml the zone inhibition value is 10±1.0cm.

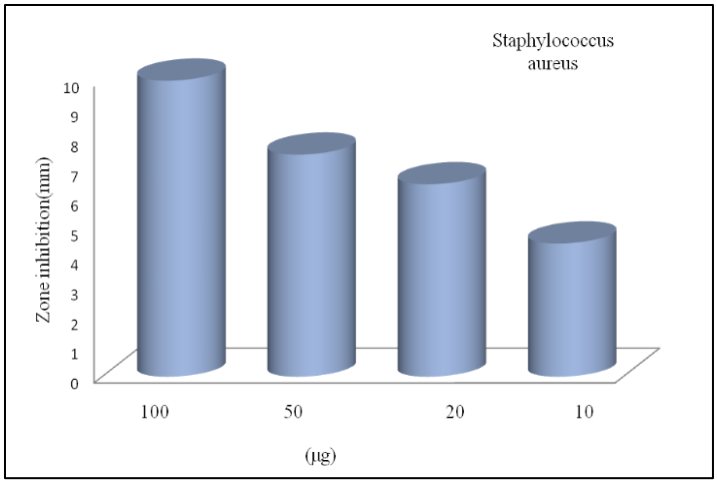

Fig.9 bar graph of Staphylococcus aureus (100, 50, 20, 10) µg/ml

Propionibacterium acnes

Antibacterial analysis done by using Propionibacterium acnes bacteria fig 10 shows the photograph of the effect of the sample against these bacteria.

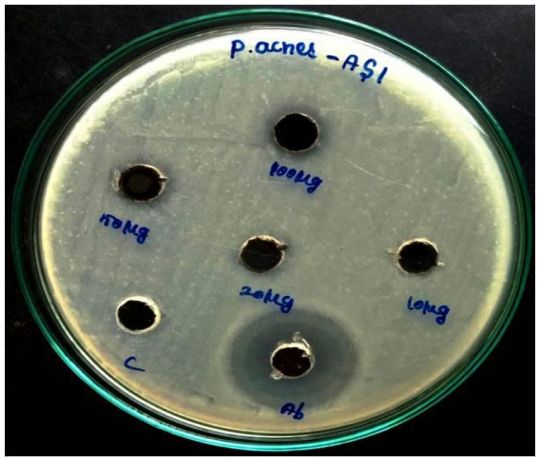

Fig.10 Effect of sample AS1 against propionibacterium acnes

Bar diagram 11 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. The control value **AB= 12.5±1.5.** The control of AB is used for **Gentamicin antibiotic.**

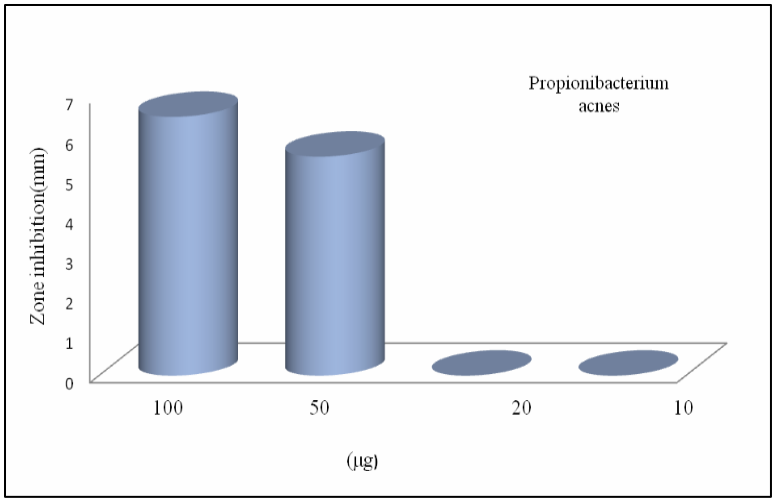

Fig.11 bar graph of Propionibacterium acnes (100, 50, 20, 10) µg/ml

4.6.2.5 E. coli

Antibacterial analysis done by using E. coli bacteria fig 12 shows the photograph of the effect of the sample against these bacteria.

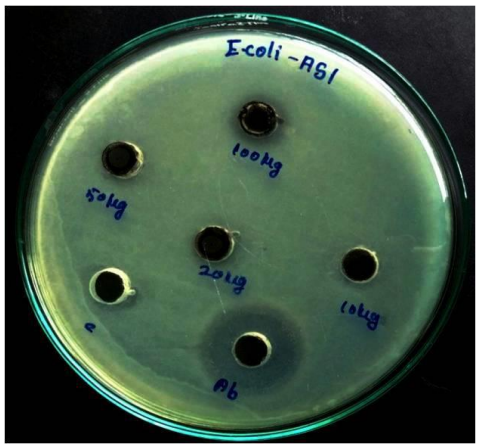

Fig.12 Effect of sample AS1 against E. coli

Bar diagram 13 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. The control value **AB= 11±1.0.** The control of AB is used for **Gentamicin antibiotic.**

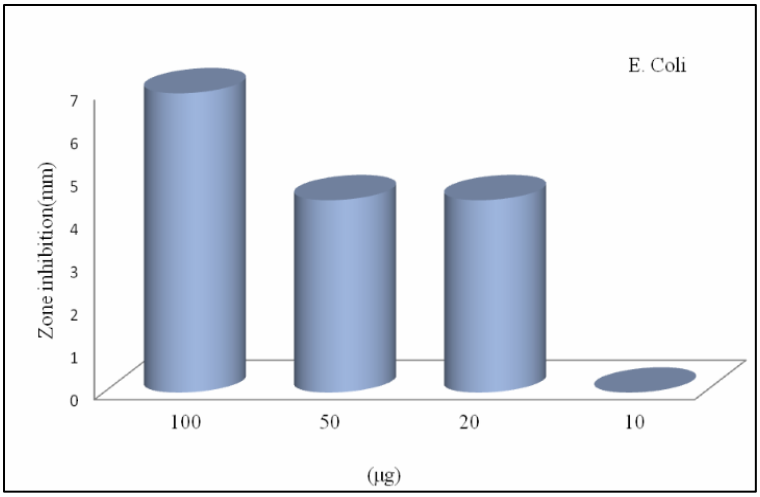

Fig.13 bar graph of Propionibacterium acnes (100, 50, 20, 10) µg/ml

Table 2.Zone of inhibition value for antibacterial analysis

S.NO	Name of the test organism	Name of the test sample	Zone of inhibition (nm)SD ± Mean				
			100 µg/ml	50 µg/ml	20 µg/ml	10 µg/ml	AB
1.	Pseudomonas aeruginosa	AS1	5.5±0.5	0	0	0	10.5±1.5
2.	Streptococcus oralis		8±1.0	4.5±0.5	4.5±0.5	0	11±1.0
3.	Staphylococcus aureus		10±1.0	7.5±1.5	6.5±0.5	4.5±0.5	10.5±2.5
4.	propionibacterium acnes		6.5±0.5	5.5±0.5	0	0	12.5±1.5
5.	E. Coli.		7±1.0	4.5±0.5	4.5±0.5	0	11±1.0

Pseudomonas aeruginosa bacteria the lower concentration they are not respond, but higher concentration there will be respond, but the value is very low compared to the control. Streptococcus oralis low concentration there will not be respond, but the concentration value is increases the inhibitation value is increases. Propionibacterium acnes low concentration there will not be respond, but higher concentration there will be respond. E.Coli the low concentration there will not be respond but higher concentration there will be respond. The prepared nickel selenide sample is good alternative for curing Staphylococcus aureus bacteria with respect to Gentamicin.

Antifungal studies

An antifungal medication, also known as an antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athletes foot,ringworm, candidiasis, serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by doctor prescription, but a few are available OTC.

Aspergillus Niger

Antifungal analysis done by using Aspergillus Niger fungal fig 14 shows the photograph of the effect of the sample against these fungal.

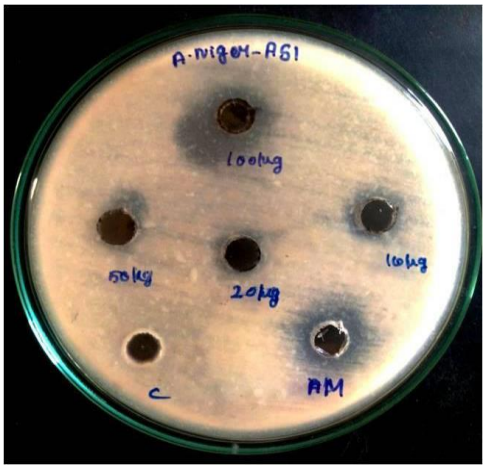

Fig: 14 Effect of sample AS1 against Aspergillus Niger

Bar diagram 15 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. Control value, **AB=11.5±1.5.** The control of AB is **Amphotericin B**. The zone of inhibition value increases with increase in concentration. The value is more for

higher concentration i.e., 100µg/ml the zone inhibition value is 13±1.0cm and other other three concentration is lower

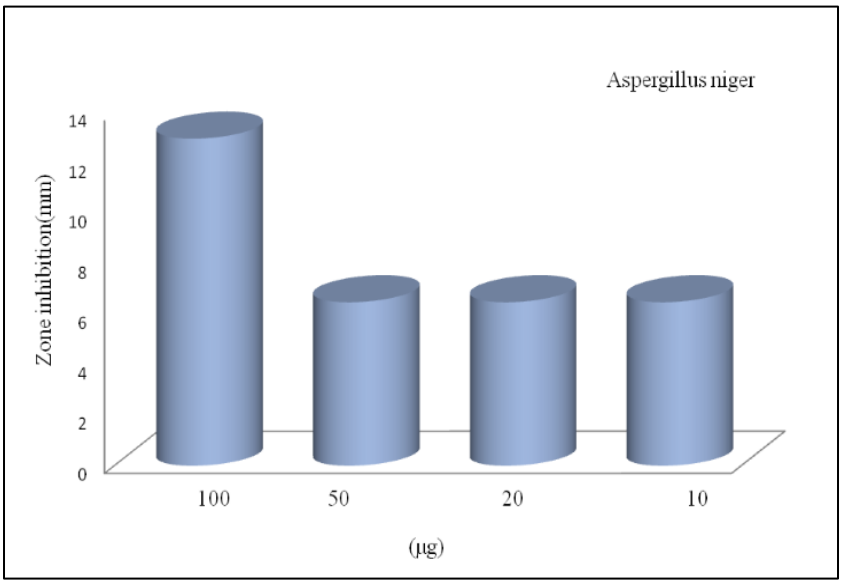

Fig. 15 bar graph of Aspergillus Niger (100, 50, 20, 10) µg/ml

Aspergillus fumigatus

Antifungal analysis done by using Aspergillus fumigatus fungal fig 16 shows the photograph of the effect of the sample against these fungal.

Fig: 16 Effect of sample AS1 against Aspergillus fumigatus.

Bar diagram 17 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. Control value **AB=10±1.0.** The control of AB is **Amphotericin B**

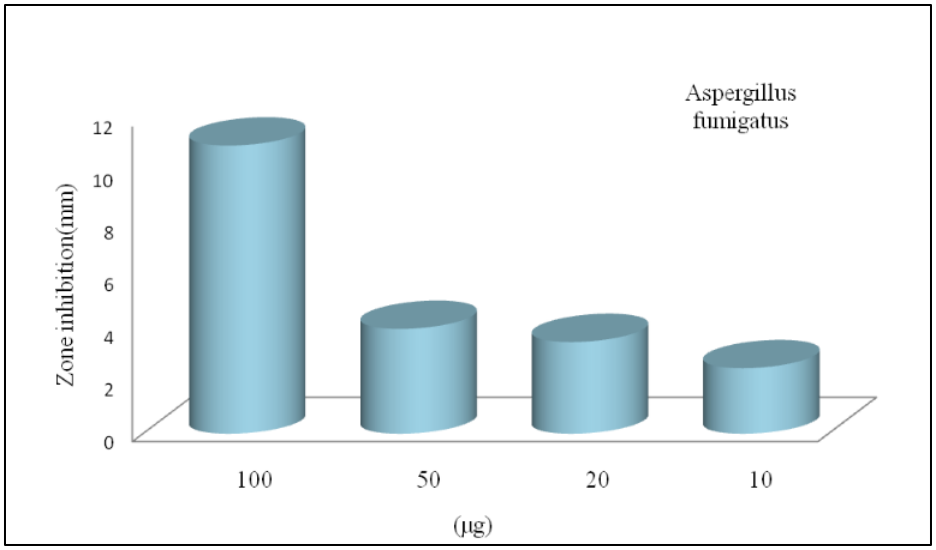

Fig.17 . bar graph of Aspergillus fumigatus (100, 50, 20, 10) µg/ml

4.7.3 Aspergillus flavus

Antifungal analysis done by using Aspergillus flavus fungal fig 18 shows the photograph of the effect of the sample against these fungal.

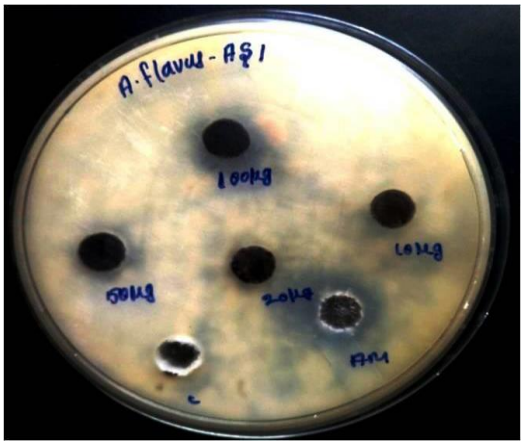

Fig: 18 Effect of sample AS1 against Aspergillus flavus.

Bar diagram 19 shows the value of concentration in the X-axis and zone of inhabitation is the Y-axis. Control value **AB=12±1.5.** The control of AB is **Amphotericin B**

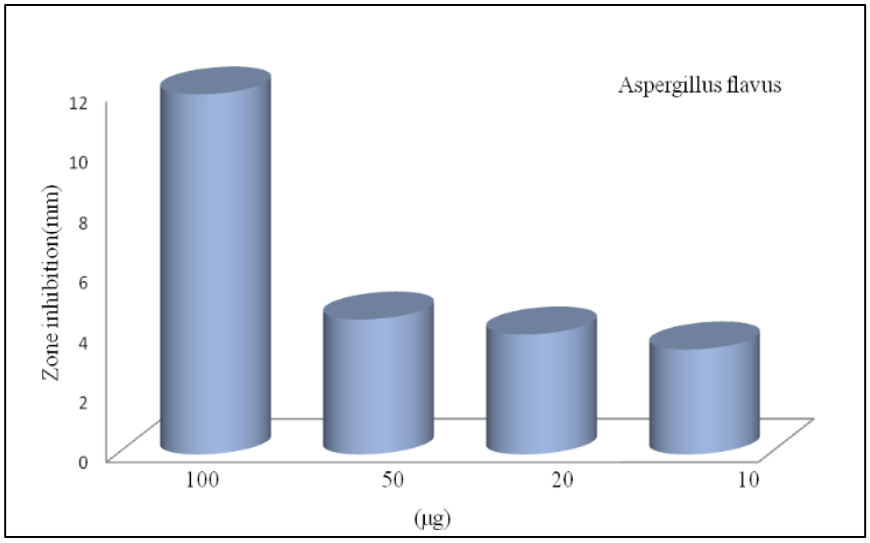

Fig.. 9 Bar graph of Aspergillus flavus (100, 50, 20, 10) µg/ml

Table .3 zone of inhibition value for antifungal analysis

S.NO	Name of the test Micro organism	Name of the test sample	Zone of inhibition (nm)SD ± Mean				
			100 µg/ml	50 µg/ml	20 µg/ml	10 µg/ml	AM
1.	Aspergillus niger	AS1	13±1.0	6.5±0.5	6.5±0.5	6.5±.5	11.5±1.5
2.	Aspergillus fumigatus		11±1.5	4±1.5	3.5±.5	2.5±.5	10±1.0
3.	Aspergillus flavus		12±1.0	4.5±0.5	4±0.5	3.5±.5	12±1.5

The Prepared nickel selenide nanosamples react very well with these three fungals such as, Aspergillus Niger, Aspergillus fumigates, Aspergillus flavus. "Aspergillus Niger", the lower

concentration, as the concentration is increase the zone of inhibitation is also increases, and higher concentration around 100µg the zone of inhibitation value is larger than that of the control. Aspergillus fumigates the low concentration the zone of inhibitation is low and higher concentration the zone of inhibitation is high. Aspergillus flavus is same as that of aspergillus fumigates.Therefore, the prepared nickel selenide nanoparticle using lemon extract is the good alternative quire of all this three fungals. The good result for antifungal studies using citrus Limon extract.

Summary and discussion

$NiSe_2$ was synthesized by green synthesis method using citrus Limon leaf extract. This influence of various parameters viz. stirring temperature, concentration of citrus Limon extract and calcinations temperature were also checked and contributions were optimized for the synthesis of $NiSe_2$ nanoparticles. The $NiSe_2$ were synthesized by green synthesis method using green chemistry. The use of plant extracts for making metallic nanoparticles is inexpensive, easily scaled up and environmentally benign. It is especially suited for making nanoparticles that must be free of toxic contaminants as required in therapeutic applications. The plant extract based synthesis can provide nanoparticles of a controlled size and morphology. In medicine, nanoparticles are being used as antimicrobial agents in bandages, for example, Applications in targeted drug delivery.

From the powder XRD analysis, the **average crystalline size** of the prepared nanoparticle is around **21.67nm.**. The **dislocation density** of prepared sample is around **0.0021295.** The peaks of different peaks are assigned by using the FTIR analysis. From the SEM analysis, the particles were agglomeration on the surface of the nanoparticles. From EDAX, the result of Ni and Se_2 and its composition was analysis in EDAX. The antibacterial analyses by using five bacteria such as, E.Coli, Pseudomonas aeruginosa, Streptococcus oralis, Staphylococcus aureus and propionibacterium acnes. From the result given "Staphylococcus aureus" the zone of inhibitation is high when sufferer to that of the other bacteria's and also the zone of inhibitation level are increases with the lower concentration to higher concentration.The antifungal analyses by using three fungal such as, Aspergillus Niger, Aspergillus fumigates Aspergillus flavus. For all the

three fungals the zone of inhibition increases with lower concentration to higher concentration and at higher concentration around 100µg its value is higher than that of the control. Therefore it is the best alternative of all three fungals.

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