



## Green synthesis of nanoparticle analysis in *Alangium salvifolium* leaves extract towards its antibacterial activity

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### Abstract

The modern scientific technology of close eco-friendly development alternative to synthesis of nanoparticles is grate movement among researchers. Present investigation achieved to the biological synthesis of antibacterial activity analysis effect of silver and zinc nanoparticles against human pathogens using *Aalangium salvifolium*. The silver and zinc nanoparticles were characterized evolved several analytical techniques UV-Visible Spectrometer, FT-IR, X-ray diffraction pattern analysis and SEM- Scanning electron microscope with EDX- analysis. The biosynthesis silver and zinc nanoparticles were determined for their antimicrobial activity against human pathogenic microorganisms. Further investigation of biosynthesized silver and zinc nanoparticle in *Aalangium salvifolium* leaves extract. The *K. pneumonia* and *S. aureus* is highest zone of inhibition than that against *E. coli* and *E. faecalis*. The moderate activity of *E.coli* and *E. faecalis* maximum activity in both samples. Whereas in zinc nanoparticles screened antibacterial activity against *K. pneumonia* and *E. faecalis* is higher zone of inhibition than that against *E. Coli*, *S aureus* *Aalangium solvifolium* leaves zinc extract. The maximum activity of *E.coli* and *E. faecalis* in both samples. The biological synthesis of silver and zinc nanoparticle is a refreshing and cost-efficient approach coming over harmful Nano synthesis techniques. The nanoparticles synthesized using *Aalangium salvifolium* own stiff inhibitory effect that valuable part to pharmacological connectedness.

**Keywords:** *Aalangium salvifolium*, UV-visible spectrometer, scanning electron microscope, microorganisms, *K. pneumonia*, *E. coli*, *E. faecalis*

### Introduction

Cornaceae are generally distributed in northern temperate regions. Economic importance includes cultivated ornamentals, such as Cornus (e.g., *C. florida*, flowering dogwood) and some timber and edible fruit trees. The Cornaceae are distinctive in being trees, shrubs, or rhizomatous herbs, with simple, usually opposite and undivided leaves, a usually cymose inflorescence, sometimes in heads, with showy, petaloid *bracts* in some taxa, the flowers usually biserial and bisexual, epigynous, generally 4-5-merous, with an intrastaminal annual disk and inferior ovary, the fruit usually a multilocular drupe (Michael G. Simpson) <sup>[1]</sup>. Eugene Wilson *et al* <sup>[2]</sup> the materials used by the Siddhars as drugs could be classified into herbal (Mooligai or thavaram), inorganic substances (Thathu) and animal products (Jeeva vaguppu). In Tamil literature, the word Marunthu (drug) itself means or denotes scented root or leaf. In general, among the Indian Systems of Medicine, Siddha uses herbs prominently. Great emphasis is given to herbs by our ancient Siddhars. It is stressed by the Siddha sutra as follows: "Ver paru thazhai paru minginikal Mella mella parpa chenduram pare" Accordingly, it is advised to administer first pure herbs in the form of liquid, powder, pill or paste. If it is not controlling, then, the physicians use mixture of herbs, metals, minerals and animal products in addition to herbs.

In view of this uniqueness, there was a dire need to identify the medicinal plants prescribed in Siddha literature and standardize the herbal drugs for some refractive diseases, and to make the international community aware of such distinctions. In the pursuit of such a venture, arthritis, the

commonly affecting disease afflicted by many people due to change in food habits, life style, etc. was selected for the study. In spite of the efficiency of eradicating both rheumatoid arthritis as well as osteoarthritis in Siddha System of Medicine, it is not popular other than Tamil Nadu. Siddha system primarily uses herbal products as well as animal and mineral matters, too. As the system is started and practiced in the Tamil speaking area, it had no opportunity to spread out until and otherwise, the most useful medicine in this system is brought to light in other languages, the effective drugs of this system may not be enjoyed by others. Moreover, such outreach in a particular language may not be possible for everyone. So, this review will be of great use is not only establishing the historical continuity but also to spread the knowledge on such drugs to the regions of other than Tamil Nadu. According to evidence of uses in the plant *Alangium salviifolium* (Linn. f) Wang. Azhinjil Cornaceae Root, bark oil is used for joints pain

Recently published ethnopharmacological studies regarding the genus Cornus (Cornaceae) have shown that among 65 species only two of them have a long tradition in a medicinal use. Cornus mas L. native to southern Europe and southwest Asia, known as cornelian cherry, is used since ancient times. Ma *et al* <sup>[3]</sup> it was used as a traditional Chinese medicine for more than 2,000 years to promote a healthy liver and kidney, and is a common ingredient of many TCM prescriptions Liu X. *et al* <sup>[4]</sup> however, much more not widely known iridoids, such as morroniside, as well as tannins were detected particularly in fruits of *C. officinalis*. The referred studies of biological activity of both

species indicate their antidiabetic and hepatoprotective properties. Based on the available reports antihyperlipidemic and anticoagulant activity seems to be unique for extracts of *C. mas* fruits, whereas anti osteoporotic and immunomodulatory activities were assigned to preparations of *C. officinalis* fruits. In conclusion, the comparison of phytochemical composition of fruits from both species revealed a wide range of similarities as well as some constituents unique for cornelian cherry or Asiatic dogwood. Thus, these phytochemicals are considered the important factor determining the biological activity and justifying the use of *C. mas* and *C. officinalis* in the traditional European and Asiatic medicine Monika E. Czerwinska [5] Recently green synthesis of nanoparticles has potential applications in environmental and biomedical fields. Green synthesis aims in particular at decreasing the usage of toxic chemicals. For instance, the use of biological materials such as plants is usually safe. Plants also contain reducing and capping agents. Here we present the principles of green chemistry and we review plant mediated synthesis of nanoparticles and their recent applications. Nanoparticles include gold, silver, copper, palladium, platinum, zinc oxide and titanium dioxide Sapana Jadoun [6]. Globally, food spoilage caused by microorganisms still widely affects all types of food and causes food waste and loss, even in developed countries. It has been estimated that the yearly losses of global food reach up to 40% due to various factors including spoilage by microorganisms Gustavsson *et al* [7]. Once these microorganisms reach food products, they grow by utilizing the nutrients and produce metabolites that cause food spoilage Parlapani *et al* [8]. Foodborne disease is another pervasive food safety problem caused by consumption of contaminated food products, which has been a significant safety concern to public health Kirk *et al* [9]. Kalem *et al* [10]. Therefore, new eco-friendly methodologies are required to reduce the growth of pathogenic bacteria and prolong the shelf-life of food products, without using chemical preservatives. Recently, many researchers investigated the possible utilization of some plant extracts as

effective natural preservatives Clarke *et al* [11]. Traditionally, the crude extracts of different parts of medical plants, including root, stem, flower, fruit, and twigs, were widely used for treatments of some human diseases Khan *et al* [12]. Antimicrobial activities of some plant species have been widely researched. For example, the crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria Castro *et al* [13]. In addition, it has been reported that the extracts from Chinese chives and cassia can effectively reduce the growth of *Escherichia coli* and other bacteria during storage of meat, juices, and milk Mau *et al* [14]. In a similar study, Doddanna *et al* [15] investigated the effect of some plant extracts on the growth of *Candida albicans*, the results indicated that the alcoholic extract of curry leaves effectively inhibit the growth of *C. albicans* with  $24.05 \pm 0.07$  after 48 h. Moreover, Nzeako *et al* [16] reported that thyme oil extract could decrease the growth of *C. albicans* and *Pseudomonas aeruginosa*.

### Plant material

The plant *Alangium salvifolium* (Tamil vernacular name- Alanji) belongs to the family cornaceae was at Andikkarai is a small village of Salem District, Tamil Nadu, India. Herbarium specimens were prepared and taxonomic identification of the plant was confirmed at the Padmavani arts and Science College for Women, Herbarium and Salem-11. A voucher specimen of Plant was deposited to that the Herbarium for future reference.

### Scientific classification

Kingdom: Plantae  
Division: Tracheophytes  
Class: Angiosperms  
Order: Cornales  
Family: Cornaceae  
Genus: *Alangium*  
Species: *A. Salvifolium*



Fig 1: Herbarium *Alangium salvifolium* Habitat of *Alangium salvifolium*

### Green Bio-Synthesized Silver and Zinc Nanoparticles Chemicals

Silver nitrate ( $\text{AgNO}_3$ ), Zinc Sulphate ( $\text{ZnO}$  NPs) was

purchased from Sigma-Aldrich Chemical Ltd. (St Louis, MO, USA) and another chemicals AR grade Hi Media, Mumbai, India.

### Preparation of plant extracts

Plant parts such as (100g) leaves were extracted separately in Soxhlet apparatus using aqueous solvent respectively. The extract were filtered through a cotton followed by Whatman filter paper No.1 and then concentrated by using a rotary evaporator 40 - 50 °C and reduced pressure to get 4.80 g yield from aqueous fractions respectively. The extracts were preserved in airtight containers and kept at 4°C until further use. The extracts were tested for using UV, FTIR, XRD, EDAX, SEM AND antimicrobial activity.

### UV-Visible spectral analysis of bio reduction of silver and zinc sulphate synthesis plant extract

The bio reduction of Silver and Zinc sulphate in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml of de-ionized water and subsequently measuring UV-visible spectra of the resulting diluents. UV-visible spectroscopy analyses of Silver and Zinc nanoparticles produced were carried out as a function of time needed for bioreduction at room temperature on Thermo Heyios 2 model spectrophotometer at 190 – 1100 nm.

### FT-IR analysis of bio-synthesis for silver and zinc plant extract

A pellet for infrared (IR) analysis was obtained by carefully grinding 2 mg of Silver and Zinc bio-synthesis plant extract with 200 mg of dry potassium bromide, ground well in mortar under an IR lamp for 30 mins and then pressing in a mold. The IR spectrum of Silver and Gold nanoparticles plant extract from 400 to 4000  $\text{cm}^{-1}$  was obtained using a Perkin-Elmer spectrum GX.

### X-RAY diffraction XRD analysis

The particle size and nature of silver, zinc sulphate nanoparticle were determined using XRD. This was carried out using Shimadzu XRD-6000 / 6100 model with 30 kv, 30mA with  $\text{CuK}\alpha$  radians at  $2\theta$  angle. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finally ground and average bulk composition is determined. The particle or grain size of the particles on the silver and zinc sulphate nanoparticles was determined using Debye Scherrer's equation  $D = 0.94 \lambda / B \cos \theta$

### EDAX spectrometer analysis of silver and zinc sulphate nanoparticles

SEM is type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan patterns. In this experiment after the synthesis of nanoparticles using the plants and then lyophilisation was done using VIRTIS BENCHTOP machine. SEM and EDAX analysis was done using JEOL-MODEL 6390 machine.

Thin film of a sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the films on the SEM grid allowed to dry by putting it under a mercury lamp for 5 minutes.

### SEM analysis of silver and zinc sulphate nanoparticles

Scanning electron microscope was done in Hitachi S-3500 N. By drop coating, silver and zinc sulphate nanoparticle were prepared for High-resolution scanning electron

microscope analysis on to pure Titanium coated. The film on the SEM grids were allowed to stand for 2 min following which the extract solution was removed using a blotting paper and grid was allowed to dry, prior to the measurement. SEM measurement performed on a Hitachi S-3500 N use these conditions 20,000 X magnification, ~15 mm working distance. Instrument operated at a 25 KV accelerating voltage, objective aperture # 3 and condenser lens strength set to 50.

### Antimicrobial Property in Bio-Synthesized Silver and Zinc Nanoparticles

#### Collection of micro organisms

The microbial strains employed in the biological assays were bacteria strains: *Klebsiella pneumoniae* (MTCC 3040), *Escherichia coli* (MTCC 119), *Enterobacter faecalis*, *Salmonella aureus* (MTCC 734) obtained from Microbial type culture collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India.

#### Antimicrobial activity of synthesized nanoparticles

Antimicrobial activity was analyzed with synthesized silver and zinc nanoparticles by well diffusion method against bacterial strains: *Klebsiella pneumoniae* (MTCC 3040), *Escherichia coli* (MTCC 119), *Enterobacter faecalis*, *Salmonella aureus* (MTCC 734) microorganisms. The pathogenic culture were cultured in broth for antibacterial assay. Approximately 8 mm diameter of well was made on Muller Hinton Agar plate with gel puncture. The cultures were swabbed on test media. Four agar wells were made on nutrient agar and each well was loaded with 1mM of AgNPs solution and ZnNPs with concentration were inoculated to the well and then the plates were incubated at 37 °C for 24 hours the zone of inhibition was observed respectively.

### Results

#### Green biosynthesis of silver and zinc sulphate nanoparticle in *Alangium salvifolium* leaves extract

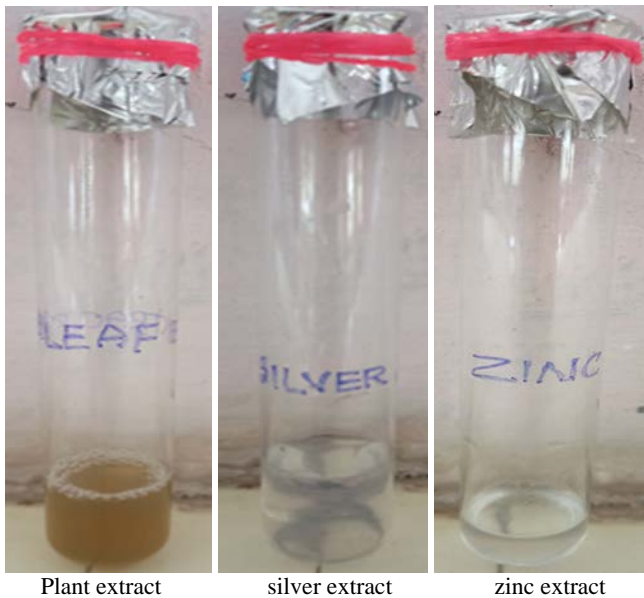
##### UV – Vis spectra analysis

The formation of Silver and Zinc sulphate nanoparticles by reduction of the aqueous silver and Zinc ions during exposure of *A. salvifolium* extract under microwave irradiation may be easily followed by UV-Vis spectroscopy. The reduction of silver ions into silver particles during exposure to the plant extract was followed by color change from leaf extract yellow colour to change colouration dark brown in color and the reduction of Zinc ions particles during exposure to leaves plant extract was followed by colour change from yellow to pale yellow in colour. It is well known that silver nanoparticles exhibit yellowish to dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. As the plant extract was mixed in the aqueous solution of the silver ions complex, it started to change the color from yellowish to brown due to reduction of silver ion and Zinc ions which may be the indication of formation silver nanoparticles. The UV-Vis spectra recorded from the *A. salvifolium* leaves plant extract synthesized silver and gold nanoparticle reaction is presented. UV-analysis has shown a higher peak value of 266 nm indicates the silver nitrate solutions and Zinc higher peak value 265. Contrary, silver and Zinc nanoparticles showed the maximum peakvalue (266-265 nm) summarized in Fig: 4

**FTIR-analysis**

For the FTIR absorption spectra of the *Aalangiium salvifolium* leaves extract leaves after bio reduction of silver and zinc nanoparticle extract. The suggest information regarding the chemical change in bio reduction can be assessed. The infrared spectra usually have sharp features that are characteristic of specific groups of molecular vibrations, making the spectra useful for sample identification. The band between 650 and 1750 cm-1 related variation of corbohydrates, lipids and proteins. Proteins are the largest group and the peptide group which is the structural repeat unit in proteins, gives upto 9 characteristic band. Amide I and amide II bands are two major bands of the protein infrared spectrum. Amide I is the most intense absorption band in proteins.

It is primarily governed by the stretching vibrations of the C = O (70-85%) and C-N groups (10-20%). It frequency is found in the range between 1600 and 1700 cm-1. The exact band position is determined by the backbone conformation and the hydrogen bonding pattern. Amide II is found in the 1510 and 1580 cm-1 region and it is more complex than amide I.

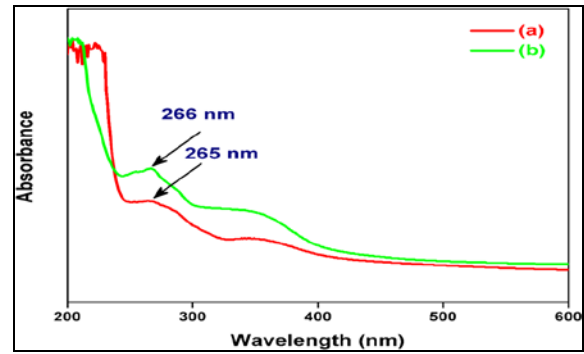


**Fig 2:** A. salvifolium leaves plant extract, Silver extract and Zinc extract.

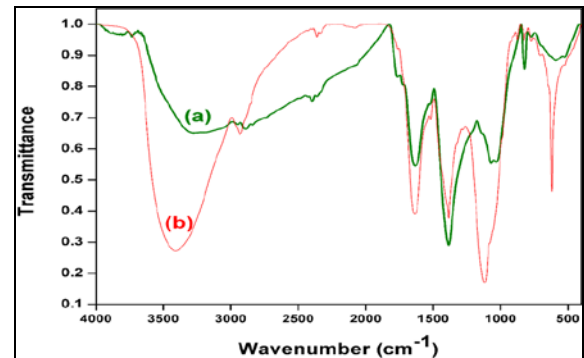


Synthesis of silver extract      Synthesis of zinc extract

**Fig 3:** Synthesis of silver and zinc in Alangiium salvifolium



**Fig 4:** UV-Vis absorption spectra of aqueous Silver nitrate (AgNPs- a) and Zinc sulphate (Zno NPs- b) with *Aalangiium salvifolium* leaves extract time intervals.



**Fig 5:** FT-IR Spectroscopy analysis of *Aalangiium salvifolium* synthesis Silver and Zinc leaves extra

Amide II derives mainly from in-plane N – H bending (40 – 60% of the potential energy). The rest of potential energy arises from the C-N (18-40%) and the C-C (about 10%) stretching vibrations.

Amide III is very complex band dependent on the details of the force field. The nature of side chains and hydrogen bonding. Therefore this band is only of limited use for the extraction of structural information. The FTIR spectra of the pure lipid compounds are more complex. Three distinct absorption bands are apparent, of which the CH<sub>3</sub> and CH<sub>2</sub> (3, 025 – 2, 954 cm-1) and the C = O ester (1, 746 cm-1, 654 cm-1) are most characteristic for lipids. From these individual lipid spectra, it is clear that characteristic and distinct finger prints for triglycerides and phospholipids exist in the FTIR spectrum. The spectra of strong bands at 1024, 1080 and 1150 cm-1 is characteristic of the C – O stretching vibrations of carbohydrate. The phosphate group in nucleic acids shows a strong peak at 1084 and 1225 cm-1. The FTIR analysis of exopolymeric substances reveals the different functional groups among the exopolysaccharides. The presence of different groups of exopolysaccharides detected by FTIR was provided in Table 1 and Figure 5

**Table 1:** FTIR Functional group of *Aalangiium salvifolium* before and after silver and zinc synthesized extract.

S.no	Groups	Silver (NM)	Zinc (nm)
1	Chloroalkanes		658
2	CH <sub>2</sub>	704	
3	Aromatic ether		
4	C-O-H Arylalyl asymmetrical		1385
5	CO	1639	1638
6	Methylene		
7	C-H	2075	2113
8	Alkynes		
9	Phosphines		
10	OH or N-H group	3418	3342

The IR spectrum of partially *Aalangiium salvifolium* leaves extract leaves silver and zinc nanoparticles showed broad intense absorption band at 621, 694  $\text{cm}^{-1}$  indicating the presence of chloroalkanes and sharp intense band at 709 – 717  $\text{cm}^{-1}$  strongly suggesting methylene ( $\text{CH}_2$ ) vibrations. Secondary alcohols, phenols and aromatic ethers characteristic bands 1232  $\text{cm}^{-1}$  were found in *Aalangiium salvifolium* leaves extract silver and zinc synthesized nanoparticles respectively. The C-H stretching vibrations were observed at 1371 to 1385  $\text{cm}^{-1}$  for all exopolysaccharides. The bending of OH in primary alcohol was observed 1411  $\text{cm}^{-1}$ . The intramolecular OH sharp bands were noted at 3398 to 3435  $\text{cm}^{-1}$ . The presence of alkynes at a characteristic band appearance at 2113  $\text{cm}^{-1}$  was noted in all the *Aalangiium salvifolium* leaves extract synthesized nanoparticle. All the spectra has shown the presence of carbohydrates, proteins, DNA and lipids in varying composition and quantity as evidenced by the appearance of differences in both shape and absorbance intensity of *Aalangiium salvifolium* leaves extract synthesized silver and zinc nanoparticles.

### XRD studies

Figure 3 showed the XRD confirming the existence of silver colloids in the 30.8, 60.5 and 78. The bragg reflections were observed in the XRD pattern at  $2\theta = 30.8, 60.5$  and 78. These bragg reflections clearly indicated the presence of sets of lattice planes and further on the basis that they can be

indexed as face-centered-cubic (FCC) structure of silver. Whereas the bragg reflections were observed in the XRD pattern 10.2, 20.2, 20.8 and 30.3. These bragg reflections clearly indicated the presence of sets of lattice planes and further on the basis that they can be indexed as face-centered-cubic structure of zinc. Hence XRD pattern silver and zinc nanoparticles formed in this present synthesis are crystalline in nature.

In addition to the bragg peaks representative of FCC silver and zinc nanoparticles, additional as yet unassigned peaks were also observed suggesting that the crystallization of bioorganic phase occurred on the surface of the nanoparticles.

### EDAX analysis essay

EDAX spectra recorded from the silver nanoparticles were showing in figure.5 EDAX spectra it is clear that silver nanoparticles reduced by *Aalangiium salvifolium* have the weight percentage of silver 41.53. Whereas EDAX spectra it is clear zinc nanoparticles reduced have the weight 43.53.

### SEM morphological characteristics of studies

SEM technique was employed to visualize the size and shape of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was 10nm and zinc in the SEM analysis that the average size was 10nm.

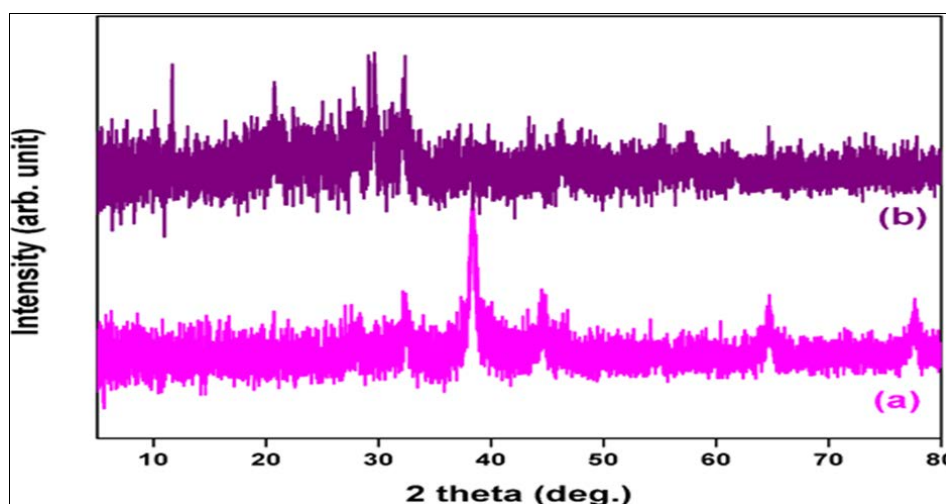


Fig: 6 Phase matched XRD pattern of silver (a) and zinc (b) nanoparticles.

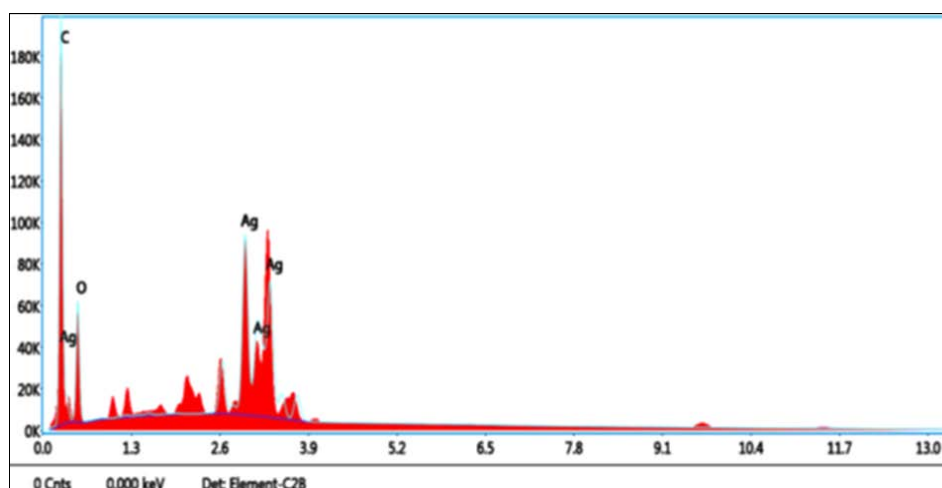
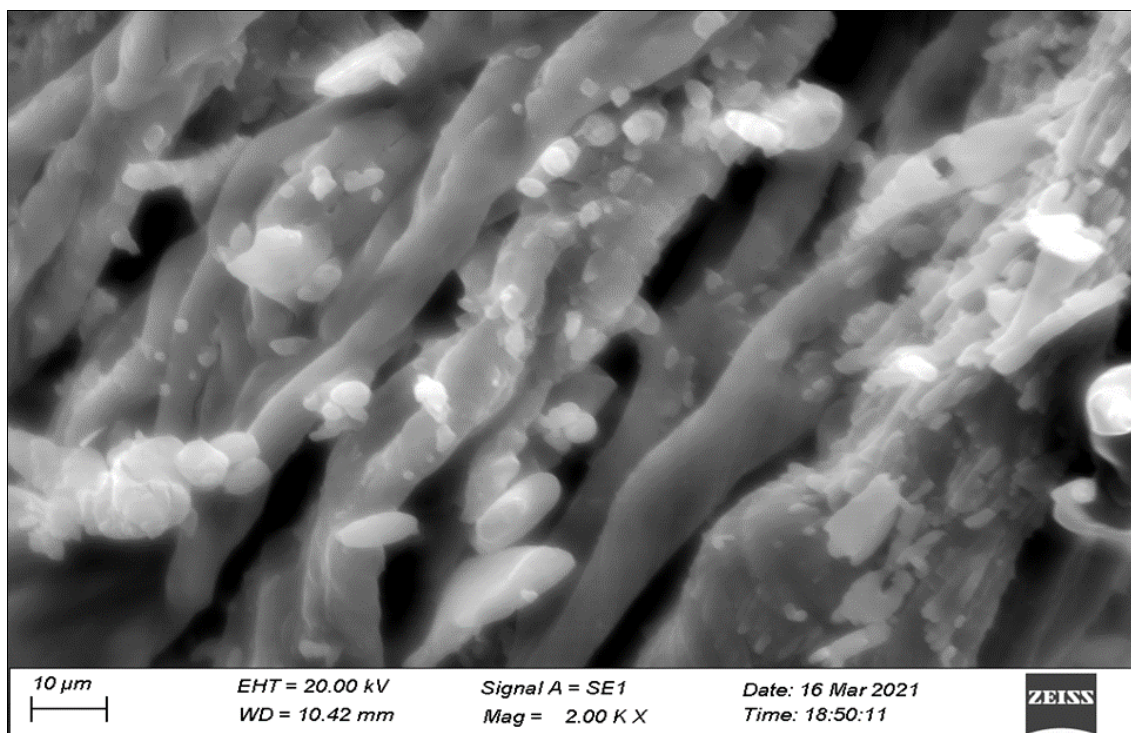


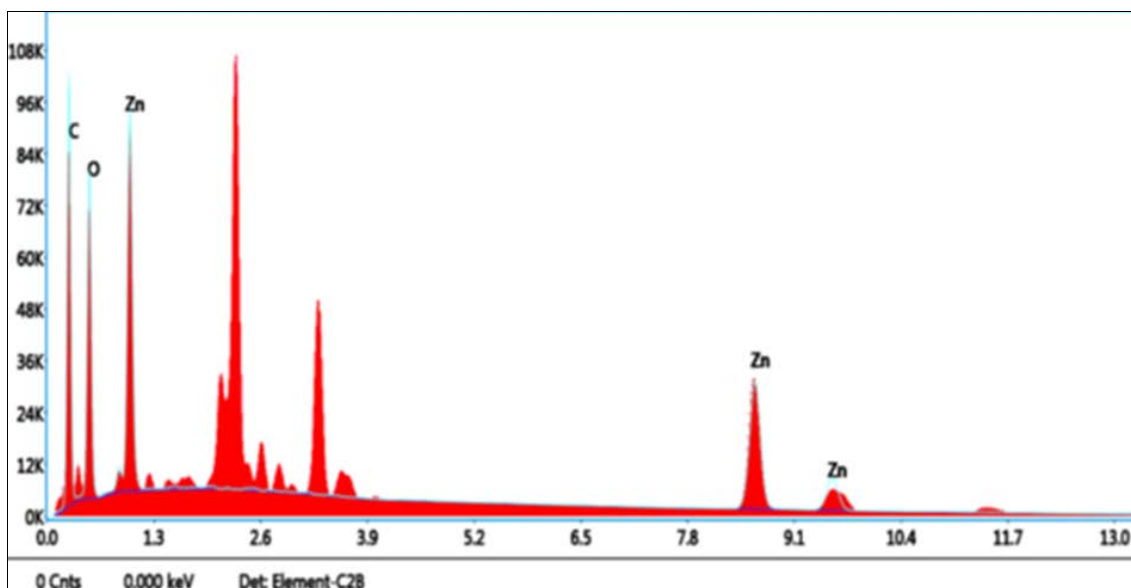
Fig 7: EDAX spectra recorded from a film after formation of silver nanoparticles X-ray emission peaks labelled

**Table 2:** Synthesis of silver nanoparticle element weight, atomic and error analysis.

Elements	Weight %	Atomic %	Error %
CK	30.98 %	55.08 %	3.79 %
OK	27.5 %	36.7 %	9.46 %
Ag	41.53 %	8.22 %	1.8 %



**Fig 8:** SEM image of silver nanoparticles formed *Aalangium salvifolium*



**Fig 9:** EDAX spectra recorded from a film after formation of zinc nanoparticles X-ray emission peaks labelled

**Table 3:** Synthesis of zinc nanoparticle element weight, atomic and error analysis.

Elements	Weight	Atomic	Error
CK	37.14	62.27	8.5
OK	19.32	24.32	8.48
Ag	43.53	13.41	2.01

**Antimicrobial Activity of Silver and Zinc Nanoparticle**  
 Antimicrobial activity of biosynthesized silver and zinc nanoparticle in *Aalangium salvifolium* leaves extract. The

*K.pneumonia* and *S.aureus* is highest zone of inhibition than that against *E. Coli* and *E, faecalis*, in *Aalangium salvifolium* plant leaves silver extract.

The moderate activity of *E. coli* and *E, faecalis* maximum activity in both samples. Where as in zinc nanoparticles showed antibacterial activity against *K. pneumonia* and *E. faecalis* is higher zone of inhibition than that against *E. coli*, *S. aureus* *Aalangium salvifolium* leaves zinc extract. The maximum activity of *E. coli* and *E. faecalis* in both samples. Table 4, 5

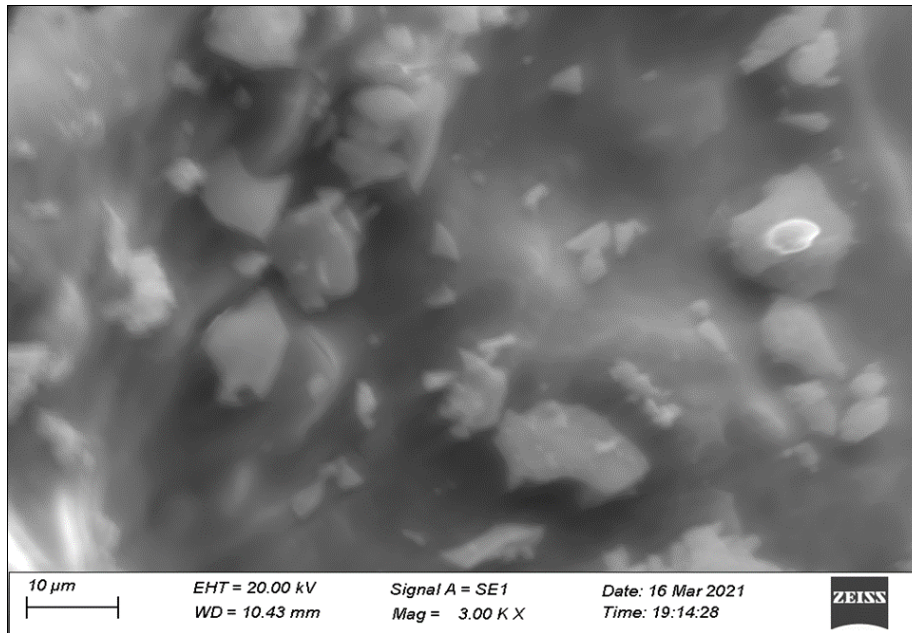


Fig 10: SEM image of zinc nanoparticles formed *Aalangium salvifolium*

Table: 4 Antimicrobial activity of silver nanoparticles against human bacterial pathogens

Ag NPs	<i>E. coli</i>	<i>E. faecalis</i>	<i>S. aureus</i>	<i>K. pneumonia</i>
5 ml	5mm	-	5mm	6mm
10ml	5mm	5mm	6mm	5mm
PE	-	-	5mm	5mm
AB	10mm	10.5mm	11.5mm	10.5mm



Fig 11: Antimicrobial efficacy of synthesis silver nanoparticles on *Aalangium salvifolium* leaves extract against human pathogen

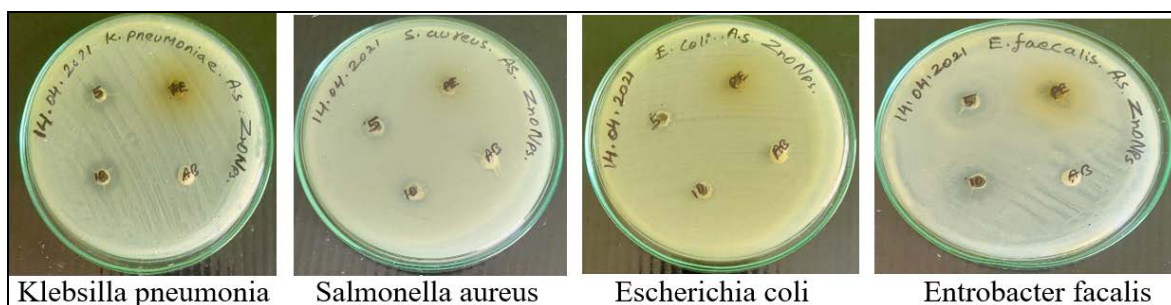


Fig 12: Antimicrobial efficacy of synthesis zinc nanoparticles on *A alangium salvifolium* leaves extract against human pathogen

Table 5: Antimicrobial efficacy of synthesis zinc nanoparticles on *Aalangium salvifolium* leaves extract against human pathogen

Zno NPS	<i>E. Coli</i>	<i>E. Faecalis</i>	<i>S. Aureus</i>	<i>K. Pneumonia</i>
5	6mm	6.5mm	5mm	7mm
10	5mm	7.5mm	6mm	7.5mm
PE	5mm	6mm	-	-
AB	-	5mm	-	-

**Discussion**

Traditional medicine has been improved in developing countries as an alternative solution to health problems and costs of pharmaceutical products. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. In the traditional system of medicine, the plant is used for various health problems and diseases. Nanotechnology is an important field of modern research dealing with synthesis,

strategy and manipulation of particle's structure ranging from approximately 1 to 100 nm in size. Within this size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk. For biomedical applications; being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions' as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities. Therefore, the aim of this to present an investigation of traditional, pharmacological and nanotechnology investigations carried out on this plant.

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