

# A NANOPARTICULATE PERSPECTIVE STUDY -BIOLOGICAL EFFECT OF NANOPARTICLE PREPARATION METHOD IN HOMOEOPATHIC MEDICINE SARSAPARILLA OFFICINALIS

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## Abstract

Nanotechnology is currently employed as tool to explore the darkest avenues of medical science in several ways. The size of nanomaterials are similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for biomedical research and applications. *Sarsaparilla* is a tropical plant from genus *Smilax*(*Smilacaceae*) found in temperate, tropical and subtropical zones worldwide. The rhizome, roots, stems and occasionally leaves of *Sarsaparilla* are used as food and traditional medicine. This plant is known to have anti-inflammatory, antioxidant, antibacterial, antifungal properties. In this study our aim was to investigate antimicrobial and antioxidant activity of the *Sarsaparilla* as nanomedicine along with its phytochemical analysis. *Sarsaparilla* mother tincture and potencies prepared based on homoeopathic law of potentiation. *Sarsaparilla* has capability of precipitating silver nanoparticle from silver nitrate at ambient temperature. The nanoparticles were characterised by UV spectrometer were the peaks obtained at 434nm for mother tincture, 251nm for 30C potency, 250nm for 200C potency and 248nm for 1M potency. SEM showed spherical shape upto 200nm size of particle and by FTIR the functional groups were investigated. The phytochemical analysis was showed positive for Flavonoids, Alkaloids, steroids, Saponin, Amino acids, Tannins, cardiac glycosides, Carbohydrate, Anthraquinone glycosides. The synthesised silver nanoparticle with *sarsaparilla* were found to be effective as antimicrobial agents against some important human pathogens like E-coli, *Staphylococcus aureus*, *Bacillus*, *Klebsiella* which cause disease and also found as a good antioxidant agent.

## Key words.

Homoeopathic medicine, silver nanoparticle, Potentiation, Fourier Transform Infrared(FTIR). Scanning electron microscopy(SEM), *Sarsaparilla*.

## INTRODUCTION

The biosynthesis of nanoparticles has become an area of great interest in recent years due to a growing need to develop environmentally friendly technology in material synthesis. The rhizome, roots, stems and occasionally leaves of *Sarsaparilla* are used as food and traditional medicine. For centuries, indigenous people around the world used the root of the *sarsaparilla* plant for treating the joint problems like arthritis, for healing skin problems like psoriasis, dermatitis and cancer(5). Additionally, they are used for relief from climacteric troubles (5). In Homoeopathic system of medicine *Sarsaparilla officinalis* has been considered as an excellent blood purifier and it is used for treating diseases especially for urinary tract infection and other diseases like Eczema, Herpetic eruptions, Emaciation, Ulcers, Rheumatism and Gonorrhoea etc(4)(9). *Sarsaparilla* tincture has prepared from its roots by Homoeopathic law of potentiation(4). Homoeopathic medicines are prepared through a characteristic process known as potentiation, where serial dilutions are performed with strong strokes at each dilutions (10). Homoeopathy is controversial because most medicines do not contain one single of the corresponding starting-substance. In this study our aim was to investigate possible nanoscience mechanism of action of Homoeopathic medicine. *Sarsaparilla* could precipitate silver nanoparticle synthesised could render a simple, fast, cost-effective and environmental friendly method.

Urinary tract infection that affects any part of urinary system, the kidneys, bladder or urethra. The most common causative organism of UTI are the gram negative bacteria of *Escherichia coli* and *Klebsiella pneumonia*(7)(8). Oxidative stress leads to cell damage and weakness of the body. In case of an inflammation it is likely that also oxidative stress can be traced in the body. Inflammation and oxidative stress reinforce themselves mutually, a vicious circle(7)(8).

*Sarsaparilla* is well indicated in kidney diseases, when symptoms are indicated scanty urine, slimy, flaky, sandy, bloody. Gravel. *Renal* colic. Severe pain at conclusion of urination. Urine dribbles while sitting. Bladder distended and tender. Child screams before and while passing urine, Sand on diaper. Dysuria in infants. Pain from right kidney downward, tenesmus of bladder, urine passes in thin, feeble stream, pain at meatus(4)(9). So there is a need to study the action of *Sarsaparilla* tincture and potencies by antimicrobial and antioxidant study and its nanomedicinal action in UTI along with its phytochemical analysis.

## MATERIALS AND METHODS.

### SYNTHESIS OF SILVER NANOPARTICLE PREPARATION:

Homoeopathic mother tincture *Sarsaparilla* was used for the synthesis of silver nanoparticles .100mg of silver nitrate was added to 20 ml of distilled water. Vigorously stirred with 5ml of mother tincture .A change in the color of solution was observed. Similarly, synthesis of silver nanoparticles were done for different potencies 30CH, 200CH and 1M of *Sarsaparilla*..

CHARACTERISATION OF SILVER NANOPARTICLES : The synthesised particles were characterized using UV spectroscopy, FTIR analysis and Scanning electron microscopy(SEM). The UV –spectroscopy analysis is used to study the absorption peak of synthesized particles. The FTIR analysis is used to study the functional groups present in the synthesized nanoparticles .The SEM technique was employed to visualize the size and shape of silver nanoparticles.

### UV SPECTROPHOTOMETER:

The reduction of pure Ag<sup>+</sup> ion was monitored by measuring the UV VIS spectrum of the reaction medium at 5 hour after diluting a small amount of the sample into distilled water , UV-VIS spectral analysis was done by using UV-VIS spectrophotometer.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) :

The fourier transform infrared spectroscopy is used to study the infrared absorption of particles . IR spectra where recorded used KBr pellets on a Perkin – Elmer GX FTIR spectrophotometer.

SCANNING ELECTRON MICROSCOPY(SEM) :

SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan patterns. SEM technique was employed to visualize the size and shape of silver nanoparticles. The pattern of dependence of the morphology and size of the nanosized objects is described.

PHYTOCHEMICAL ANALYSIS:

*Sarsaparilla* mother tincture ,6CH ,12CH, 30CH ,200CH AND 1M have been used for all studies.All reagents have been prepared following standard protocols. *Sarsaparilla* mother tincture has been used as the positive control.A preliminary phytochemical study to determine the phytoconstituents present was undertaken for the synthesised solutions and homoeopathic formulations.

ANTIOXIDANT ACTION:

Antioxidant property was determined by DPPH assay.

Dissolve 39.4mg of DPPH in ethanol or methanol =0.1mM solution .DPPH solution is highly unstable so preparation is done just before using.Ascorbic acid is used as comparison solution.Add ascorbic acid to DPPH solution and take this solution as a standard .Add medicine (*Sarsaparilla*) into DPPH solution in different test tubes. Keep this solution resting in a dark room for 30 minutes. The purple color of DPPH-sample solution will start to fade. Now compare this solution with standard. OD is measured using colorimeter and ,DPPH radical scavenging activity is measured by

$$\% \text{ of inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control.}} \times 100$$

ANTIMICROBIAL ACTION :

Preparation of microbial cultures and isolation of *E Coli* , *Klebsiella.Sp* , *Pseudomonas,Sp* , *staphylococcus.Sp* were used as test from P.G department of Biotechnology, Alva's college .And microscopic examination was done for the confirmation and were maintained in slants.

ANTIMICROBIAL ASSAY:

Petri dishes were plated with Nutrient Agar media and allowed to solidify for 30 mins. The last organism were spread on surface of the media using sterile swab stick. Cork borer (7mm) was used to bore wells in media . 20µl of medicinal and silver nanoparticle extracts in different potencies (30CH, 200CH, 1M and Mother tincture) were dispensed into the wells using a micropipette. A positive control of amoxicillin (30mcg/disc) was kept and the extract was allowed to diffuse for 1 hr at room temprature. Then the plates were incubated at 37°C for 24 hrs. Zones of inhibition were measured in centimetre scale.

**RESULT:****Synthesis of Silver nanoparticles from Homoeopathic Mother tincture of Sarsaparilla.**

The Silver nanoparticles were synthesised by the reduction of silver ions. This was shown by the change in colour of solution from golden yellow to dark brown. The colour change was observed after 25mins.

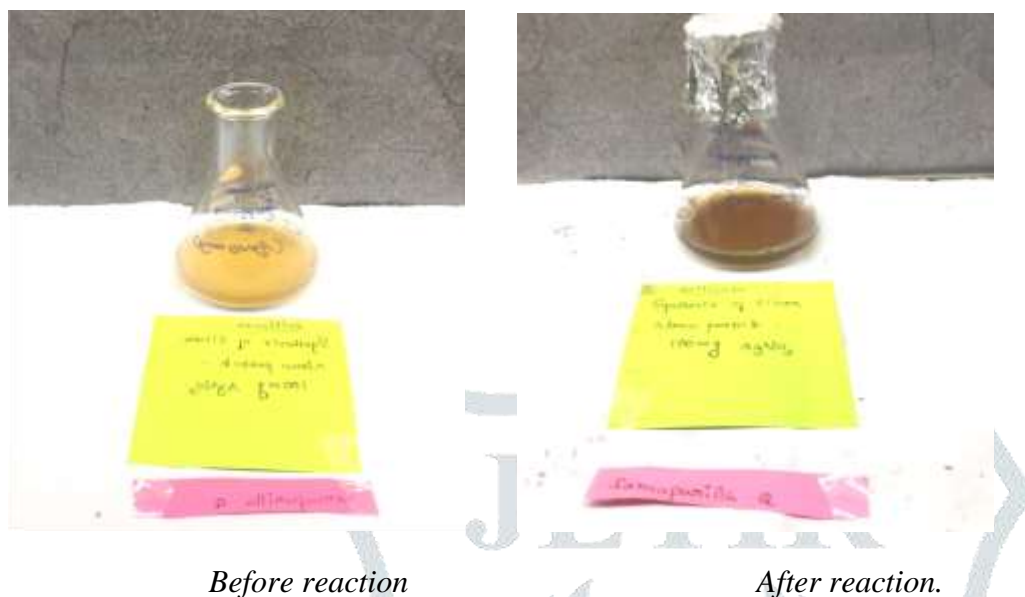


Figure 1: Colour change indicates the formation of nanoparticles.

**Characterisation of Silver Nanoparticles:****UV-Visible Spectroscopy:**

The change in the colour was visually observed which indicates the presence of silver nanoparticles. The change in the colour is mainly because of surface Plasmon resonance. The UV-visible spectroscopy is an important technique to study the metal nanoparticles.

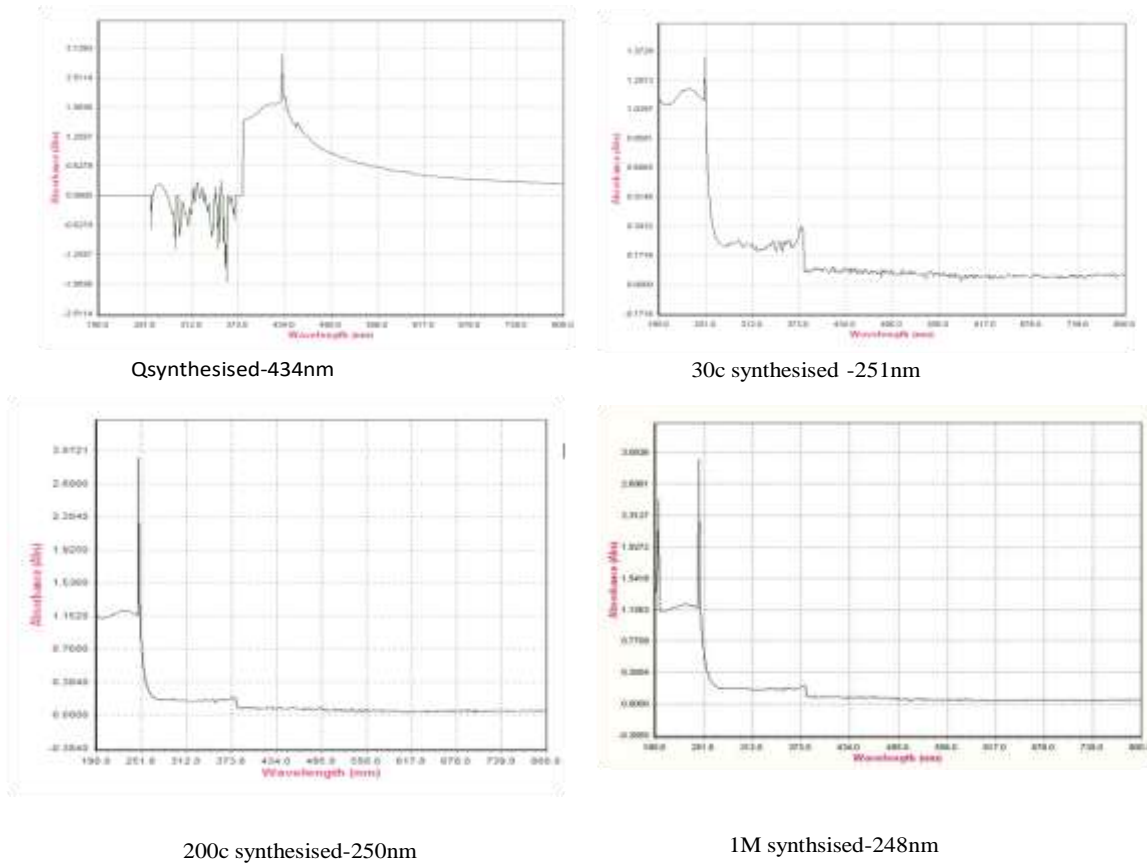


Figure 2:UV absorption spectroscopy

The above figure indicates that the absorption peak of synthesised nanoparticle prepared from *Sarsaparilla officinalis* was at the range of 434 nm for synthesised mother tincture, 251nm for 30c synthesised, 250nm for 200c synthesised, 248nm for synthesised 1M.

### Scanning electron microscope(SEM).

The scanning electron microscopy was used to observe the surface morphology. Figure 3, 4 and 5 shows particles were spherical in shape up to 200nm.

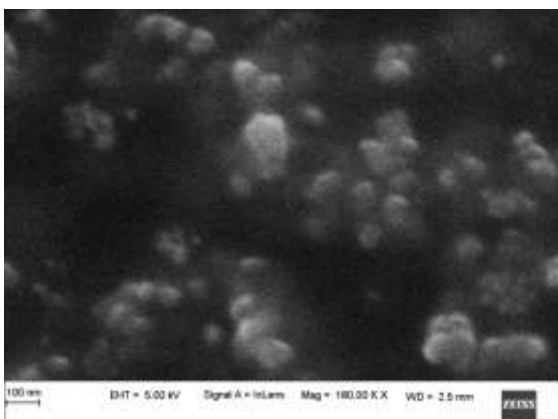


Figure 3. Synthesised with sarsaparilla

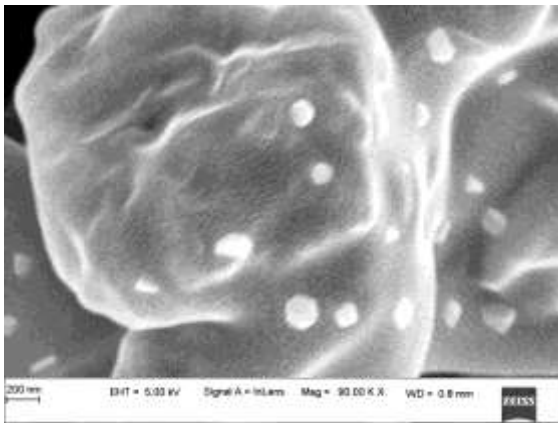
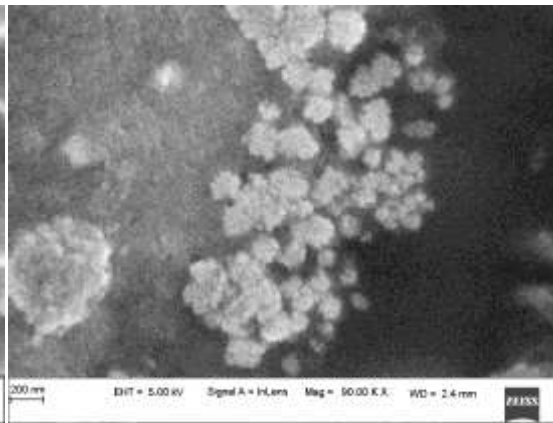
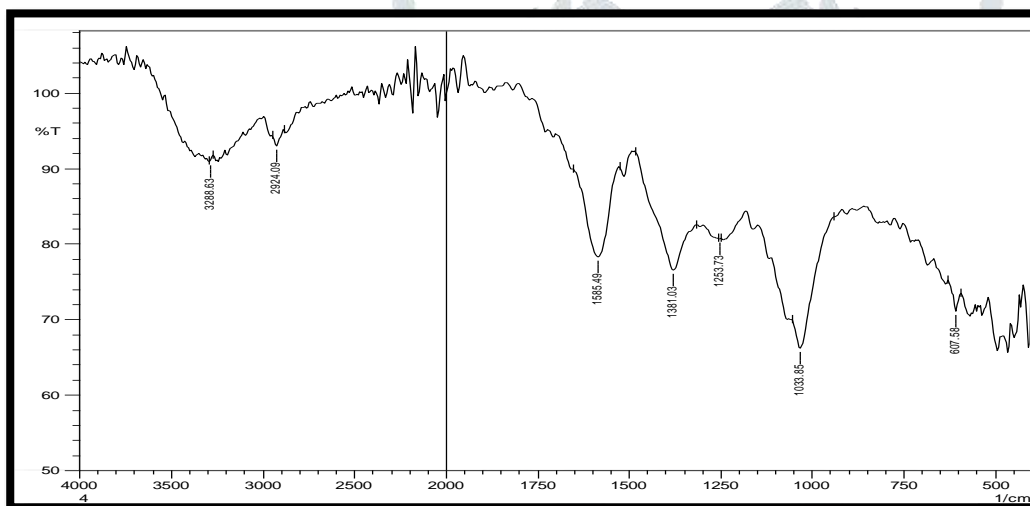
Figure 4. *Sarsaparilla* tincture

Figure 5. Synthesised with 30C

### Fourier transform infrared spectroscopy (FTIR)

The IR spectrum for sample of *Sarsaparilla* showed bands at  $567.07\text{cm}^{-1}$ ,  $1035.77\text{cm}^{-1}$ ,  $1265.30\text{cm}^{-1}$ ,  $1379.10\text{cm}^{-1}$ ,  $1583.56\text{cm}^{-1}$ ,  $2972.94\text{cm}^{-1}$ ,  $3311.78\text{cm}^{-1}$  and for SNPs  $607.58\text{cm}^{-1}$ ,  $1033.85\text{cm}^{-1}$ ,  $1253.73\text{cm}^{-1}$ ,  $1381.03\text{cm}^{-1}$ ,  $1585.49\text{cm}^{-1}$ ,  $2924.09\text{cm}^{-1}$ ,  $3288.63\text{cm}^{-1}$ .

Figure 6 .FTIR spectra of powdered *Sarsaparilla* tincture.

The band at  $1265.30\text{cm}^{-1}$  showed the presence of C-O bond. The band at  $1379.10\text{cm}^{-1}$  corresponding to C-N bond. The band at  $1583.56\text{cm}^{-1}$  showed C=C bond. The band at  $2972.94\text{cm}^{-1}$  represents C-H bond and the band at  $3311.78\text{cm}^{-1}$  represents the stretch of N-H bond.

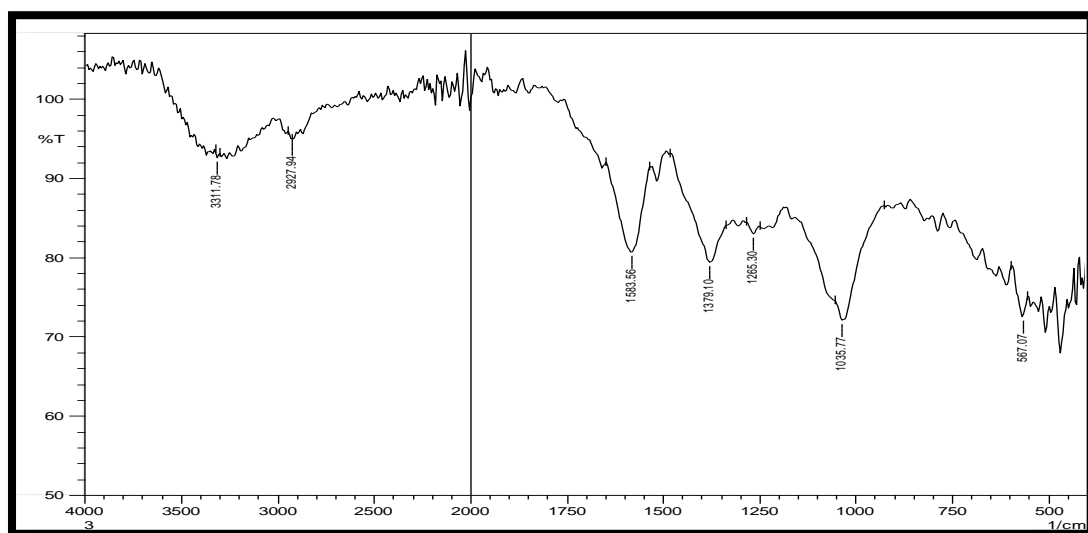


Figure 7. FTIR spectra of powdered SNPs.

The band at  $1253.73\text{cm}^{-1}$  showed the presence of C-O bond. The band at  $1381.03\text{cm}^{-1}$  represents C-N bond. The bands at  $1585.49\text{cm}^{-1}$  corresponding to C=C bond. The bands at  $2924.09\text{cm}^{-1}$  represents C-H bond and the bands at  $3288.63\text{cm}^{-1}$  showed the presence of N-H bond.

### Phytochemical Analysis:

The phytochemical analysis of sarsaparilla mother tincture, potencies and SNPs were summarised in below table 1.

| Sl.no | Phytoconstituents       | Q   | 30CH | 200CH | 1M  | Qsyn | 30syn | 200syn | 1Msyn |
|-------|-------------------------|-----|------|-------|-----|------|-------|--------|-------|
| 1     | Flavonoid               | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 2     | Alkaloid                | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 3     | Saponin                 | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 4     | Amino acid              | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 5     | Tannins                 | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 6     | Carbohydrate            | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 7     | Steroids                | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 8     | Cardiac glycosides      | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 9     | Anthraquinone glycoside | +ve | -ve  | -ve   | -ve | +ve  | -ve   | -ve    | -ve   |
| 10    | Proteins                | -ve | -ve  | -ve   | -ve | -ve  | -ve   | -ve    | -ve   |

Table 1. Phytochemical analysis with Homeopathic medicine and SNPs

Sarsaparilla mother tincture contains Flavonoids, Amino acid, Alkaloids, steroids, Saponin, Tannins, cardiac glycosides, Carbohydrate, Anthraquinone glycosides. Synthesised mother tincture contains Flavonoids, Alkaloids, steroids, Saponin, Tannins, cardiac glycosides, Carbohydrate, Anthraquinone glycosides.

Antioxidant action:

Antioxidant property was determined by DPPH assay. The antioxidant action of Sarsaparilla mother tincture, potencies and SNPs are summarised in below table 2. The percentage of inhibition most observed in synthesised 200C and least observed in 30C potency.

| SI.NO | Samples          | % of inhibition |
|-------|------------------|-----------------|
| 1     | Mother tincture  | 83.3±2%         |
| 2     | 30C              | 58.3±2%         |
| 3     | 200C             | 75±2%           |
| 4     | 1M               | 83.3±2%         |
| 5     | Synthesised Q    | 83.3±2%         |
| 6     | Synthesised 30C  | 75±2%           |
| 7     | Synthesised 200c | 91.6±2%         |
| 8     | Synthesised 1M   | 83.3±2%         |

Table 3. Antioxidant activity with Homeopathic medicine and SNPs

Antimicrobial action :

The Antimicrobial activity was done by agar well diffusion method .The zone of inhibition of *Ecoli* most observed in synthesised 30C and least observed in 30C potency. The zone of inhibition for *Staphylococcus aureus* most observed in synthesised 200C and least observed in 1M potency. The zone of inhibition for *Klebsiella* was most observed in synthesised mother tincture and least observed 1M potency.The zone of inhibition of *Bacillus* was most observed in synthesised tincture and least in 200C potency.

| SL .NO | MICRO-ORGANISM        | Q     | 30    | 200   | 1M    | Qsyn  | 30syn | 200syn | 1Msyn |
|--------|-----------------------|-------|-------|-------|-------|-------|-------|--------|-------|
| 1.     | <i>E coli</i>         | 1cm   | 0.9cm | 1cm   | 0.9cm | 1.2cm | 1.5cm | 1.2cm  | 1cm   |
| 2.     | <i>Staphylococcus</i> | 1cm   | 1cm   | 0.9cm | 0.8cm | 1cm   | 1.2cm | 1.4cm  | 1cm   |
| 3.     | <i>Klebsiella</i>     | 0.9cm | 0.9cm | 0.8cm | 0.7cm | 1.4cm | 1.1cm | 1cm    | 1cm   |
| 4.     | <i>Bacillus</i>       | 0.8cm | 0.8cm | 0.7cm | 0.9cm | 1.4cm | 1.1cm | 1cm    | 1cm   |

Table 3:Antimicrobial action with Homeopathic medicine and SNPs

CONCLUSION

Homoeopathy is controversial because most of medicines do not contain one single molecule of corresponding starting substance. Despite of all criticism Homoeopathic medicines have excellent clinical effects and widely used. Many recent studies have proved that nanotechnology has a new therapeutic

modalities in silver particles for use in medicine (1). By UV spectrometer peak we concluded that reduction has taken place in solution (1). The colour change in reaction mixture was observed through visual observation. Scanning electron microscopy showed particles were spherical in shape up to 200nm. FTIR showed presence of different functional groups. In this investigation the biosynthesised silver nanoparticle with sarsaparilla were found to be effective as antimicrobial agents against some important human pathogens like *E coli*, *Staphylococcus aureus*, *Bacillus*, *Klebseilla* which cause disease and also found as a good antioxidant agent. The phytochemical analysis was showed positive for Flavonoids, Alkaloids, steroids, Saponin, Tannins, cardiac glycosides, Carbohydrate, Anthraquinone glycosides.

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