



HOMOEOPATHY IN NANOPARTICULATE PERSPECTIVE - BIOLOGICAL EFFECT OF NANOPARTICLE PREPARATION METHOD IN HOMOEOPATHIC MEDICINE

Homeopathy

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ABSTRACT

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. In this study our aim was to investigate a possible nanoscience mechanism of action of homeopathic medicine. Homeopathic medicines are prepared through a characteristic process known as potentization. Homeopathic mother tincture *Equisetum hyemale* is capable of precipitating silver nano particles from silver nitrate at ambient temperature. In recent years UTI in females has been the one among the most recorded cases in India, Urinary tract infections are common in women, and many women experience more than one infection during their lifetime. The nanoparticles were characterised by UV spectrophotometer, were the peaks obtained are 250 for mother tincture, 217 for 200C, 203 for 15C, 201.6 for 30C, 222.6 for 30C (self potentised) respectively. AFM shows the shape and size of particles and by FTIR the functional groups in the mother tincture. The synthesized silver nanoparticles were generally found to be effective as antimicrobial agent against some important human pathogens like *E-coli*, *Pseudomonas*, *Klebsiella*, *Staphylococcus aureus* which cause disease.

KEYWORDS

Potentisation, Silver, Nano-meter, Homoeopathic medicine, Fourier Transform Infrared Spectroscopy (FTIR), Atomic Force Microscope (AFM), *Equisetum hyemale*.

INTRODUCTION

Homeopathy is a time-tested two-century old empirical system of healing. Homeopathic medicines are prepared through a characteristic process known as potentization, where serial dilutions are performed with strong strokes at each step of dilution. Homeopathy is controversial because most medicines do not contain one single molecule of the corresponding starting-substance. From the perspective of conventional medicine, all homeopathic medicines are nothing but placebo used to give some psychological relief to patients. Despite all criticism, the time-tested clinical results of homeopathy observed also in infants caused widespread popularity among users.[2]

Equisetum hyemale could precipitate silver particles from silver nitrate. The silver nanoparticles synthesised could render a simple, fast, cost-effective, and environmental friendly method. Then the biological activities of the biologically synthesised silver particle are evaluated by antibacterial activities.[3]. In recent years UTI in females has been the one among the most recorded cases in India, Urinary tract infections are common in women, and many women experience more than one infection during their lifetimes. Risk factors specific to women for UTIs include: Female anatomy. A woman has a shorter urethra than a man does, which shortens the distance that bacteria must travel to reach the bladder. So there is a need to study about increasing the efficiency of drug by addition of silver, which has an antimicrobial action.[3]

In UTI infections, the first two frequent bacteria were respectively, *Escherichia coli* and *Klebsiella pneumoniae*. The gram negative bacteria of *Escherichia coli* and *Klebsiella pneumoniae* were the most common uropathogenic bacteria causing UTI. So minimum inhibitory concentration is found by testing the antimicrobial action of Homeopathic medicine on *Staphylococcus*, *klebsiella*, *E-coli*, *pseudomonas*. [3]

Equisetum is a well indicated medicine for UTI. when symptoms indicated are severe, dull pain and feeling of fullness in bladder, not revealed by urination.[4][5][6]. frequent urging with severe pain at the close of urination, urine flows only drop by drop, sharp, burning, cutting pain in urethra while urinating.[4][6], incontinence in children, old women.[4][6], retention and dysuria during pregnancy and after delivery.[4]. Involuntary urination.[4][6], much mucus in urine.[4][5], biting and itching in meatus[4][6]. The aim of the study was to synthesize the silver nanoparticle and to evaluate the antimicrobial activity.

METHODOLOGY

Preparation of potencies

Mother tincture, potencies 30C and 200C of *Equisetum hyemale* collected from Dr. Willmar Schwabe India pvt. Ltd. 15C and 30C

potencies were made at Alva's Homoeopathic college, Mijar. 10 drops of mother tincture and 90 ml of distilled water was added to phial and 10 strokes of equal strength was given, 1st potency was prepared. To another clean phial one drop from previous and 99 drops of distilled water added and 10 equal strength stroke is given, forms the 2nd potency. Continue previous step upto 14th potency, for preparing 30 ml of 15th potency, take 0.3ml from 14th potency then add 29.7ml of dispensing alcohol (for preservation), 15th potency is prepared. From 15th potency one drop was taken and 99 drops of distilled water added and 10 equal stroke is given, it forms the 16th potency. Continue the same step till 29th potency. From 29th potency 0.3 ml is taken and 29.7ml of dispensing alcohol is added and 10 strokes are given, forms the 30th potency.

Synthesis of silver nanoparticle preparation

Homeopathic mother tincture *Equisetum hyemale* was used for the synthesis of silver nanoparticles. 100mg of silver nitrate was added to 20 ml of distilled water. Vigorously stirred with 5ml mother tincture. A change in the colour of solution was observed after 25 minutes. The extract was stored at room temperature for further use.

Characterisation of silver nanoparticles:

The synthesised particles were characterized using UV spectroscopy, FTIR analysis and AFM. The UV-spectroscopy analysis 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 AFM analysis is used to study the size of the particles.

UV Spectrophotometer

The reduction of pure Ag⁺ 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.

Atomic force microscopy

The nanoparticles were prepared by the solution and the solution was casted on to silicon wafers to make into thin films. These films were analysed in non contact mode using a specific Nanotechnology Nano-R2 instrument.

Fourier transform infrared spectroscopy (FTIR)

The Fourier transform infrared spectroscopy is used to study the infrared absorption of particles. IR spectra were recorded using KBr pellets on a Perkin-Elmer GX FTIR spectrophotometer.

Antimicrobial action:

Preparation of microbial cultures and isolation

E-Coli, *Klebsiella*, *Pseudomonas*, *staphylococcus* were used as test from P.G department of 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. The silver nanoparticle extract in different potencies (15C, 30C, 200C, Mother tincture, sac lac) 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 temperature. Then the plates were incubated at 37°C for 24 hrs. Zones of inhibition were measured.

RESULT :

Synthesis of nanoparticles from homeopathic mother tincture *Equisetum hyemale*.

The silver nano particles were synthesized by the reduction of silver ions. This was shown by the change in the colour of solution from light green to dark brown. The colour change in the reaction mixer was observed after 25 min.



(a) Before the reaction (b) After the reaction
Figure 1 : Colour change indicates the formation of nanoparticles

Characterization of synthesized 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.

Mother tincture

The figure 2 indicates that the absorption peak of synthesized nanoparticle prepared for *Equisetum hyemale* mother tincture was at range of 250nm.

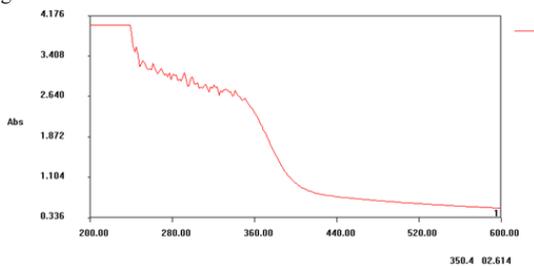


Figure 2: UV spectrophotometer for mother tincture

200c potency

The figure 3 indicates that the absorption peak of synthesized nanoparticle prepared for *Equisetum hyemale* 200 was at range of 217nm.

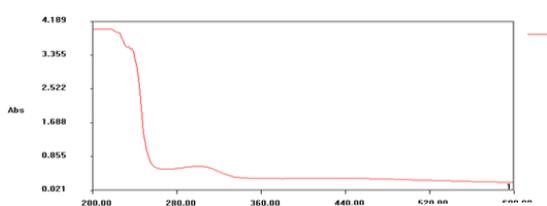


Figure 3 : UV spectrophotometer for 200C potency

15c Potency

The figure 4 indicates that the absorption peak of synthesized nanoparticle prepared for *Equisetum hyemale* 15 was at range of 203nm.

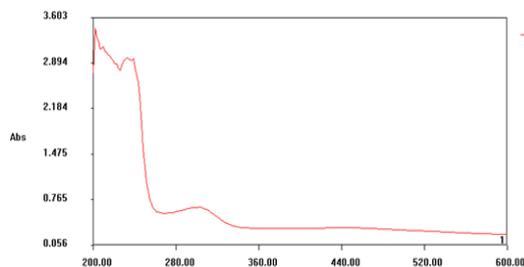


Figure 4 : UV spectrometer of 15C potency

30c potency

The figure 5 indicates that the absorption peak of synthesized nanoparticle prepared for *Equisetum hyemale* 30 was at range of 201.6 nm.

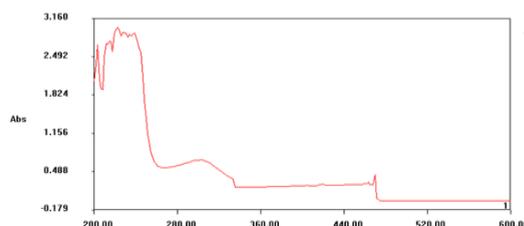


Figure 5: UV spectrometer of 30C potency

30 C potency (self potentised)

The figure 6 indicates that the absorption peak of synthesized nanoparticle prepared for *Equisetum hyemale* 30 (self potentised) was at range of 222.4 nm.

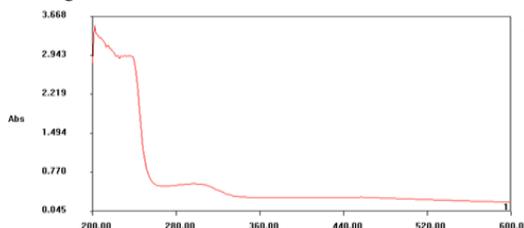


Figure 6 : UV spectrometer of 30C potency (self potentised)

FTIR :

The IR spectrum for sample of SNPs showed bands at 3422.12cm⁻¹, 2960.92cm⁻¹, 2923.04cm⁻¹, 2852.68cm⁻¹, 2428.13cm⁻¹, 2369.26cm⁻¹, 2336.88cm⁻¹, 1631.55cm⁻¹, 1571.55cm⁻¹, 1459.69cm⁻¹, 1334.29cm⁻¹, 1265.41cm⁻¹, 1168.27cm⁻¹, 1025.32cm⁻¹, 820.93cm⁻¹, 667.87cm⁻¹.

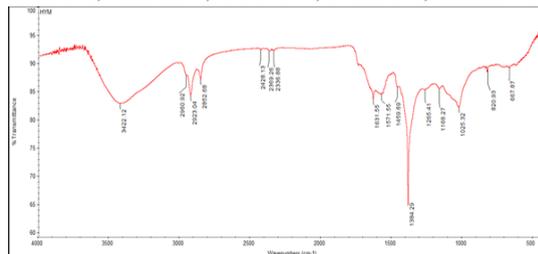


Figure 7 : FTIR spectra of powdered SNPs

The band at 3422.12cm⁻¹ represents stretch of C-H bond. The band at 2960.92cm⁻¹ corresponding to C-H bond. The band at 2923.04cm⁻¹, showed H-C=O bond. The band at 2852.68cm⁻¹ represent C-C bond, the band at 2428.13cm⁻¹ C-O bond. The band at 2369.26cm⁻¹ represents the C-H bond. The band at 2336.88cm⁻¹ showed the presence of C-O bond.

AFM

The AFM was used to observe the morphology and roughness. Figure 8 showed the particles were spherical shaped with size 265.8 nm

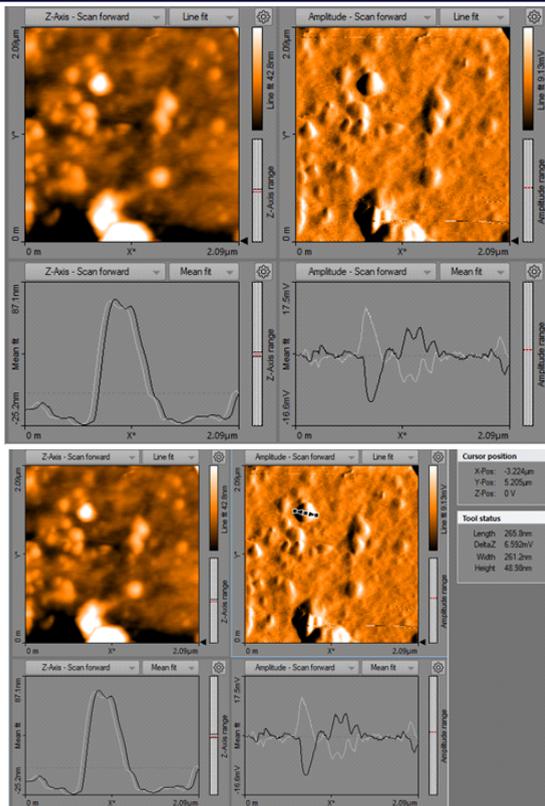


Figure 8 : Atomic force microscope image.

Antimicrobial action

The Antimicrobial activity was done by agar well diffusion method .The zone of inhibition of *E-coli* most observed in 30C (Self potentised), 15C, the zone of inhibition for *pseudomonas* most observed in mother tincture, 30C (purchased), 200C and least observed in 30C (self potentised),15C,the zone of inhibition for *staphylococcus aureus* is most observed in 30C(purchases) and least observed in mother tincture , 200C ,the zone of inhibition of *klebsiella* is least observed in 30C (purchased).

In silver nanoparticle preparation the zone of inhibition of *E-coli* least observed in mother tincture , the zone of inhibition of *staphylococcus* is observed in mother tincture, 30C,15C, the zone of inhibition of *klebsiella* is most on 30C (purchased) and least observed mother tincture , 30 C (self potentisation),15C , 200C ,the zone of inhibition for *pseudomonas* is most observed in 200C and least in 30C(purchased).

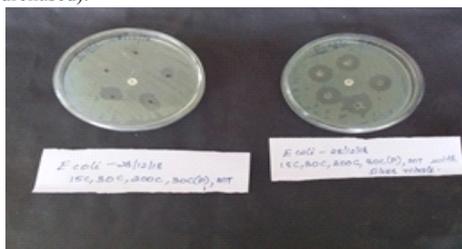


Figure 9 : Antimicrobial action of homeopathic medicine on *E coli*



Figure 10 : Antimicrobial action of homeopathic medicine on *klebsiella*



Figure 11 : Antimicrobial action of homeopathic medicine on *staphylococcus*



Figure 12 :Antimicrobial action of

Tab 1.Antimicrobial action with Homeopathic medicine

Sl.no	Micro Organism	Mother Tincture	30C	30c (purchased)	15C	200C	Amoxicillin
1.	<i>E coli</i>	0.2cm	1cm	0.7cm	0.9cm	0.7cm	-
2.	<i>Staphylococcus</i>	0.1cm	0.8cm	0.9cm	0.7cm	-	-
3.	<i>Klebsiella</i>	0.5cm	0.8cm	0.6cm	0.9cm	0.8cm	-
4.	<i>Pseudomonas</i>	1cm	0.7cm	0.9cm	0.4cm	0.8cm	-

Tab 2. Antimicrobial action with synthesised silver nanoparticles in Homeopathic medicine

Sl .no	Micro Organism	Mother Tincture	30C	30c (purchased)	15C	200C	Amoxicillin
1.	<i>E-coli</i>	0.8cm	1cm	0.9cm	1cm	0.1cm	-
2.	<i>Staphylococcus</i>	1.1cm	1.2cm	1.1cm	1.1cm	1.1cm	-
3.	<i>Klebsiella</i>	0.8cm	0.6cm	1.5cm	1.5cm	0.5cm	-
4.	<i>Pseudomonas</i>	1cm	1cm	0.7cm	1cm	1.2cm	-

DISCUSSION:

Homeopathy is controversial because most of medicines donot contain one single molecule of corresponding starting substance . Despite all criticism, the time-tested clinical results of homeopathy observed also in infants caused widespread popularity among users. In medium and high potencies, which are commonly used in clinical practice, the presence of starting-source is likely to be zero but it is „immaterial . It may be argued that what matters here is the „size of the possible encrypted information, perhaps with the electromagnetic signature of the starting-substance.(1)By UV spectrometer peaks we had come to conclusion that reduction has taken place in solution .The colour change in reaction mixture was observed through visual observation (3) . Homeopathy, a mode of therapy, was established in the late 18th century by German physician, Samuel Hahnemann. Hahnemann, during his experiments, prepared medicines from a wide variety of natural products. He discerned that the infinite dilutions of these substances carried out in steps and accompanied by vigorous shaking 'succussion' (together known as potentization) at each dilution step, elicited some kind of a potent activity to these solutions. However, a major lacuna has been the lack of evidence of physical existence of the starting material. The main difficulty in arriving at a rational explanation stems from the fact that homeopathic medicines are used in extreme dilutions, including dilution factors exceeding Avogadro's number (8). By this study using Atomic Force Microscope the size and shape of particle in nanometre has been obtained from 15th potencies and by FTIR, the functional groups in homeopathic medicine Equisetum are studied. Antimicrobial action of homeopathic medicine equisetum is studied on organism. S.aureus, pseudomonas, Klebsella , Ecoli, and a positive result with transparent circumference around the wells with potencies has been observed, which conformed that the bacterias were unable to grow in and around the homeopathic medicines. Also many recent study have proved that nanotechnology has proved a new therapeutic modalities in silver

particles for use in medicine (7). In this investigation the biosynthesized silver particles were shown to have antibacterial effects (7). Cell line study showed the action of *E. histolytica* on UTI.

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