

"HOMOEOPATHY IN NANO-PARTICULATE PERSPECTIVE -SYNTHESIS OF SILVER NANOPARTICLE AND EFFECT OF HOMOEOPATHIC MEDICINE CURCUMA LONGA INCLUDING IT'S ANTI OXIDANT AND ANTIMICROBIAL ACTIVITY"

Homeopathy

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ABSTRACT

Nanotechnology serves as one of the most capable technologies and accomplished approaches of the 21st century which is safe and efficacious for novel drug delivery systems. In recent decades, homeopathy has developed a growing basic science, clinical, and health services research literature showing unique physicochemical properties, biological activity in vitro and in vivo, good real-world effectiveness and exceptional safety. Recent studies reveal that homeopathic remedies contain nanoparticles (NPs) of source materials formed by "topdown" mechanical grinding in lactose and/or succussion (forceful agitation) in ethanolic solutions (potentization). Homoeopathic mother tincture(Q) and potencies 6CH, 12CH, 30CH, 200CH & 1M was used for this entire study. In this study our aim was to investigate the augmentation of therapeutic potential of *Curcuma longa* (Turmeric) (Q), potencies and its application in a Nano perspective. *Curcuma longa* Linn commonly known as Haldi, family Zingiberaceae is an important cultivated medicinal crop of India. Its use in medication, culinary and cosmetics is well known since centuries. Initially silver nanoparticles (SNP'S) were synthesised from the mother tincture of *Curcuma longa* homoeopathic medicine. Characterizations of nanoparticles were done using different methods, which included ultraviolet-visible spectroscopy, where the silver nanoparticles showed an absorption peak at around 350nm for mother tincture(Q), 250nm for 6CH, 248nm for 12CH and 30CH, 250nm for 200CH and 249nm for 1M. SEM shows that shape of the particles are spherical and size was upto 100nm, FTIR-has shown the different functional groups and the IR spectrum for sample of *curcuma longa* (Q) has shown bands at 563.21cm⁻¹, 1786.96cm⁻¹, 1033.86cm⁻¹, 1267.23cm⁻¹(C-O), 1379.10cm⁻¹(C-N), 1581.63cm⁻¹(C=C), 2922.16cm⁻¹(C-H), 3026.91 cm⁻¹(C-H) and for (Q) SNP's 574.79cm⁻¹, 823.60cm⁻¹, 1033.86cm⁻¹, 1242.16cm⁻¹(C-O), 1371.39cm⁻¹(C-N), 1593.20cm⁻¹(C=C), 2901.80cm⁻¹(C-H), 3013.71 cm⁻¹(C-H). Through this study it was evident that, the synthesised nanoparticles were found to be effective as a potent anti oxidant and antimicrobial agent against pathogens like *E.coli*(ATCC 25922), *Klebsiella*.Sp, *Staphylococcus aureus* (ATCC 29213), *Lactobacillus acidophilus* (MTCC 10307) which are causative of many diseases. The present study also establishes that homoeopathic drug solutions are not mere placebo liquid but an unlimited source of nano particles and quantum dots derived from the original drug substance.

KEYWORDS

Nanoparticles, Silver nanoparticles, Homoeopathic medicine, Potentization, *Curcuma longa*, Antimicrobial activity, Antioxidant property, UV spectrophotometry, Fourier Transform Infrared Spectroscopy(FTIR), Scanning electron microscopy (SEM).

INTRODUCTION

Homeopathy is a more than 200-year-old whole system of complementary and alternative medicine (CAM). [2] In recent decades, homeopathy has developed a growing basic science, clinical, and health services research literature showing unique physicochemical properties, biological activity in vitro and in vivo, good real-world effectiveness, exceptional safety, and, in many studies, cost-effectiveness. [3]

Homeopathic medicines are prepared by a pharmacological process of Potentisation/trituration in any of the three scales of potencies: Decimal(X), Centesimal (C) and Millesimal (LM). [4] Each dilution step divides the original quantity by 10, 100 and 50,000 respectively. Recent studies reveal that homeopathic remedies contain nanoparticles (NPs) of source materials formed by "topdown" mechanical grinding in lactose and/or succussion (forceful agitation) in ethanolic solutions (potentization). [2]

Reducing particle size increases surface area. Nanoparticles are nano sized particles (1 to 100 nanometers diameter) having a large surface area to volume ratio which gives them different properties from those bulk forms of the same material. The body runs on micro and nanodoses. The levels of hormones and cell signaling agents in our body is in the picograms/millilitre range such as 10–900 pg/ml for estradiol, 300–10,000 pg/ml for testosterone, and 8–27 pg/ml for T4. These small amounts are known to have a profound effect on the human body. [4]

Homeopathy is in fact, a form of nano-pharmacology, medicine prepared through a specific pharmacological process of potentisation/trituration. Trituration (repeated grinding with lactose powder) is carried out by pulverisation in a grinding machine. This results in breaking down of medicinal substance into nanoparticles. Nanoparticles have biological activity. Nanoparticles in homeopathic medicines exert biological effects on the body. Nanoparticles can cause hormesis. Hormesis is an adaptive response that is induced by stimulation at low doses and inhibition at high doses. [4] Silver nanoparticles apart from the numerous applications that they have, also shows anti bacterial and anti fungal properties. [5]

Curcuma longa, Turmeric (Rhizome)-Southern Asia belongs to the natural order of Zingiberaceae. Curcumin from turmeric, as well as other substances in this herb, have anti oxidant properties. [6] Turmeric has long been used in both Ayurvedic and Chinese medicine as an anti inflammatory, to treat digestive disorders and for the liver problems and the treatment of skin diseases and wound healing [6][7]. Several medical properties have been attributed to *Curcuma longa* Linn. Rhizome of Haridra is known to possess therapeutic activities and has been used by medical practitioners as an anti-diabetic, hypolipidemic, anti-inflammatory, anti-diarrhoeal, hepatoprotective, anti-asthmatic and anti-cancerous drug. Haridra is widely used in cosmetology due to its tyrosinase inhibition (anti-melanin) activity. [8] Curcumin has potential in the treatment of various forms of cancer, including prostate, breast, skin and colon. [9]

The objective of this study is to understand the nanoscience mechanism of action of Homoeopathic medicine from mother tincture(Q) and potencies (6CH, 12CH, 30CH, 200CH, 1M) of *Curcuma longa* and to investigate the augmentation of its therapeutic potential and its application in a Nano perspective and to understand its Anti-microbial activity of minimum inhibitory concentration (MIC) and Anti-oxidant properties.

MATERIALS AND METHODS.

SYNTHESIS OF SILVER NANOPARTICLE:

Homoeopathic mother tincture *Curcuma longa* (Purchased from Willmar Schwabe India Pvt.Ltd) 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.

CHARACTERISATION OF SILVER NANOPARTICLES:

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

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.

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 was described.

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.

PHYTOCHEMICAL ANALYSIS:

Curcuma longa Q, 6CH, 12CH, 30CH, 200CH AND 1M have been used for all studies. All reagents have been prepared following standard protocols. *Curcuma longa* mother tincture has been used as the positive control. A detailed 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 (*Curcuma longa*) into DPPH solution in different test tubes. Keep this solution resting in a dark room for 30 minutes. The purple colour 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

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

Preparation of microbial cultures and isolation of *E. coli* (ATCC 25922), *Klebsiella*.Sp, *Staphylococcus aureus* (ATCC 29213), *Lactobacillus acidophilus* (MTCC 10307) were used from Alva's Centre For Research In Nanotechnology. 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 minutes. The 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 extract in different potencies (6CH, 12CH, 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 temperature. Then the plates were incubated at 37°C for 24 hrs. Zones of inhibition in mm were measured.

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

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 20mins.

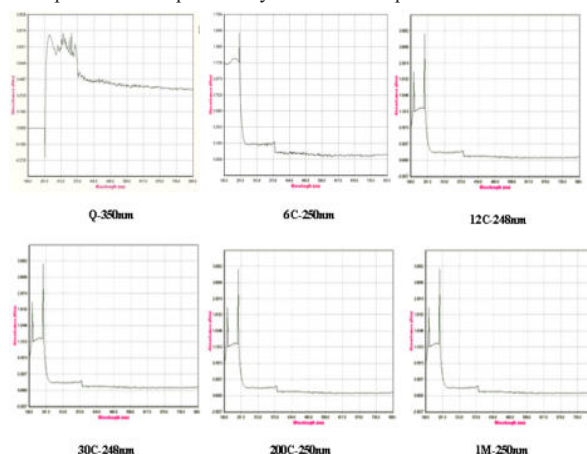


(a) Before the reaction.

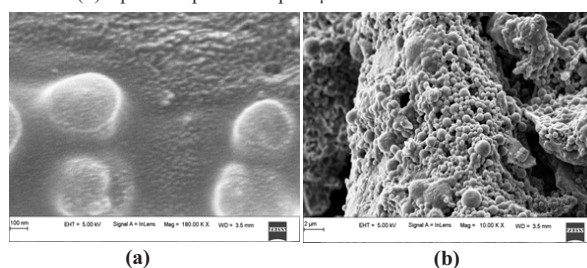
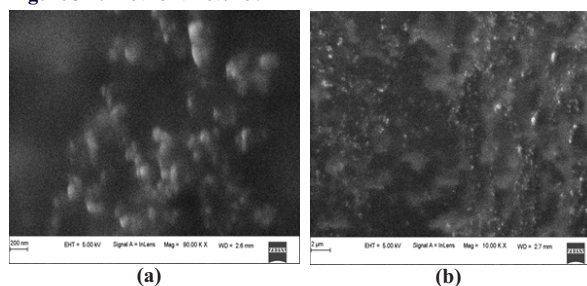
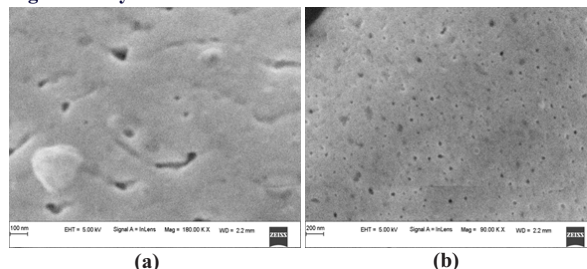
(b) After the reaction.

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 PEAK OF SYNTHESISED SNP'S OF *Curcuma longa*.****Scanning Electron Microscopy(SEM):**

The size and shape of the particles assessed by SEM is summarized as: (Fig-3(A)) (a)- Spherical particles upto 100nm. (b)-Spherical particles upto 2µm (Fig-3(B)) (a)- Spherical particles upto 200nm. (b)- Spherical particles upto 2µm. (Fig-3(C)) (a)- Spherical particles upto 100nm. (b)-Spherical particles upto 2µm.

**Figure 3A: Mother tincture :****Figure 3B: Synthesised Mother tincture :****Figure 3C: Synthesised 6C Potency:****Fourier Transform Infrared Spectroscopy (FTIR):**

The IR spectrum for sample of *Curcuma longa* (Q) has shown bands at 563.21cm⁻¹, 786.96cm⁻¹, 1033.86cm⁻¹, 1267.23cm⁻¹, 1379.10cm⁻¹, 1581.63cm⁻¹, 2922.16cm⁻¹, 3026.91cm⁻¹.

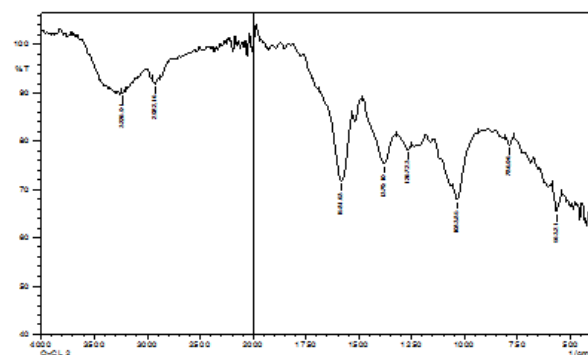


Figure 4a: FTIR SPECTRA OF POWDERED Q.

The band at 1267.23cm^{-1} represents stretch of C-O bond. The band at 1379.10cm^{-1} corresponds to C-N bond. The band at 1581.63cm^{-1} represents C=C bond. The band at 2922.16cm^{-1} shows the presence of C-H bond, The band at 3026.91cm^{-1} indicates presence of C-H bond.

The IR spectrum for sample of *Curcuma longa* (Q) synthesised has shown bands at 574.79cm^{-1} , 823.60cm^{-1} , 1033.86cm^{-1} , 1242.16cm^{-1} , 1371.39cm^{-1} , 1593.20cm^{-1} , 2901.80cm^{-1} , 3013.71cm^{-1} .

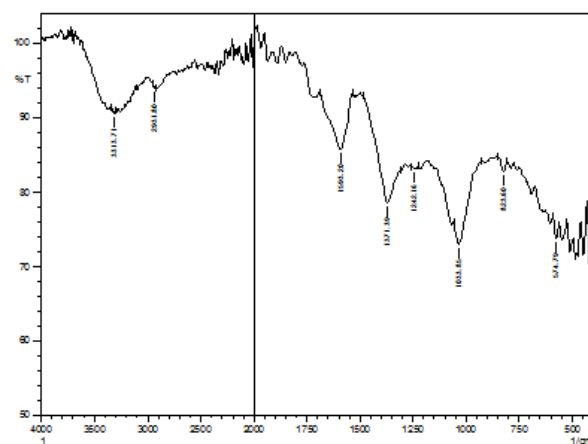


Figure 4b: FTIR SPECTRA OF POWDERED SNP'S.

The band at 1242.16cm^{-1} represents C-O bond, The band at 1371.39cm^{-1} shows presence of C-N bond. The band at 1593.20cm^{-1} indicates the presence of C=C bond. The band at 2901.80cm^{-1} represents C-H. The band at 3013.71cm^{-1} represent C-H bond.

Phytochemical Analysis:

The Phytochemical analysis of *Curcuma longa* Q and different potencies for medicines and synthesised samples were summarized in below Table 1.

Table 1:Phytochemical Analysis Result-Curcuma longa(CL).

NO.	PHYTO CONSTITUENTS	SAMPLES									
		MEDICINE					SYNTHESISED (AgNO ₃)				
		CL Q	CL 6C	CL 12C	CL 30C	CL 200C	CL 1M	SYN Q	SYN 6C	SYN 12C	SYN 30C
1	FLAVONOIDS	+	-	-	-	-	+	+	+	+	+
2	ALKALOIDS	+	+	+	+	+	+	+	+	+	+
3	SAPONINS	-	-	-	-	-	-	-	-	-	-
4	PHENOLS	+	-	-	-	-	+	+	+	+	+
5	TANNINS	-	-	-	-	-	-	-	-	-	-
6	CARBOHYDRATES	+	-	-	-	-	-	-	-	-	-
7	STEROIDS	+	+	-	-	-	+	-	-	-	-
8	CARDIAC GLYCOSIDES	+	-	-	-	-	-	-	-	-	-
9	ANTHRACENONE GLYCOSIDES	+	+	-	-	-	-	-	-	-	-
10	PROTEINS	-	-	-	-	-	-	-	-	-	-

Phytochemically all the samples has shown the presence of Alkaloids. The presence of Flavanoids and Phenols were seen in

mother tincture and all synthesised samples (Q, 6C, 12C, 30C, 200C, 1M). Carbohydrates and Cardiac glycosides were tested positive only in mother tincture. Steroids were found to be present in Q, 6C of medicinal samples and synthesised mother tincture.

Antioxidant action: Antioxidant property was determined by DPPH assay. Ascorbic acid was used as a standard to assess the photoelectric colorimetric absorbance. The percentage of Antioxidant activity observed were as given below (Table 2).

Table 2:Antioxidant Assay of Curcuma longa(CL).

Radical Scavenging Activity	Samples. Medicine.						Synthesised Snp's.					
	CL Q	CL 6C	CL 12C	CL 30C	CL 200C	CL 1M	SYN Q	SYN 6C	SYN 12C	SYN 30C	SYN 200C	SYN 1M
% Of Inhibition	83.3 ±2%	58.3 ±2%	58.3 ±2%	66.6 ±2%	66.6 ±2%	75 ±2%	75 ±2%	58.3 ±2%	58.3 ±2%	66.6 ±2%	66.6 ±2%	66.6 ±2%

Curcuma longa (CL) Q has shown the highest anti-oxidant activity of $83.3 \pm 2\%$. CL 1M and Synthesised CL Q has shown $75 \pm 2\%$ of anti-oxidant activity. $66.6 \pm 2\%$ of anti-oxidant activity was observed in CL 30C, CL 200C, SYN CL 30C, SYN CL 200C and SYN CL 1M samples. CL 6C, CL 12C, SYN CL 6C and SYN CL 12C was found to have $58.3 \pm 2\%$ of anti-oxidant activity.

Antimicrobial action :

The Antimicrobial activity was done by agar well diffusion method. The zone of inhibition of *E. coli* (ATCC 25922) was most observed in CL SYN 1M followed by CL SYN 30C and CL SYN Q. The zone of inhibition for *Staphylococcus aureus* (ATCC 29213) was most observed in CL SYN 6C and least observed in CL 1M. The zone of inhibition for *Klebsiella*. Sp was most observed in CL SYN Q and least observed in CL 12C and CL 30C. The zone of inhibition of *Lactobacillus acidophilus* (MTCC 10307) was most observed in CL 200C and was least observed in CL 12C.

Table 3:Antimicrobial action with Homeopathic medicine.

Zone of inhibition in mm (20µl)

SL. NO	MICRO- ORGANISM	CL-Q	CL 6C	CL 12C	CL 30C	CL 200C	CL 1M
1.	E coli (ATCC 25922)	10	7	6	7	7	7
2.	Staphylococcus aureus (ATCC 29213)	8	8	7	8	9	6
3.	Klebsiella. Sp	9	7	6	6	7	-
4.	Lactobacillus acidophilus (MTCC 10307)	7	7	6	8	8	-

Table 3: Antimicrobial action with synthesised silver nanoparticles in Homeopathic medicine.

SL. NO	MICRO- ORGANISM	CL-Q	CL 6C	CL 12C	CL 30C	CL 200C	CL 1M
1.	E coli (ATCC 25922)	11	10	10	12	9	13
2.	Staphylococcus aureus (ATCC 29213)	10	12	9	10	11	8
3.	Klebsiella. Sp	10	9	9	8	9	-
4.	Lactobacillus acidophilus (MTCC 10307)	10	9	9	11	14	-

DISCUSSION:

Homeopathy is controversial because most of medicines do not 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.⁽¹⁾

The drug proving experiment on healthy human being brings out large number of sign and symptoms specific to each homeopathic drug. So in effect, parallels are drawn between the disease and the drug. In homeopathy this parallel relationship between drug and disease arises at nanolevels operations of body functioning. It means that the cause of the disease as well as the cure of disease has moved away from the diagnostic and pathological levels, to the nanolevel operation of human body.⁽²⁾

By UV spectrometry peaks we had concluded that reduction has taken place in solution. The colour change in reaction mixture was observed

through visual observation⁽¹²⁾. The method of Top-Down nanofabrication discovered by Dr. Hahnemann is a physical Top-Down approach of nanotechnology in which he applied two stages. First one is the trituration and then the succussion (together known as potentization). It has always been argued that this process leads to nothingness i.e.; after 12 stages of preparation there will be only the vehicle (alcohol) remaining and there is no chance of any remnants of the original drug material. This perception got the scientific majority as per the dictums of Avogadro's number. The present study shows that irrespective of any scientific assumptions there remains nanoparticles in all the available potencies of homeopathic drugs from lower 12C to the currently available highest potency CM.⁽¹⁰⁾ By this study using SEM the size and shape of particle in nanometre has been obtained and by FTIR, the functional groups in homeopathic medicine *Curcuma longa* were studied. Antimicrobial action of homeopathic medicine *Curcuma longa* were studied on organism *E. coli* (ATCC 25922), *Klebsiella* Sp., *Staphylococcus aureus* (ATCC 29213), *Lactobacillus acidophilus* (MTCC 10307) and a positive result with transparent circumference around the wells has been observed, which confirmed that the bacterias were unable to grow in and around the synthesised medicines. Also this study shows that nanotechnology has proved a new therapeutic modalities in silver. The present study also establishes that homeopathic drug solutions are not mere placebo liquid but an unlimited source of nano particles and quantum dots derived from the original drug substance.

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