



“PHYTOCHEMICAL ASSAY OF BIOSYNTHESISED GOLD AND SILVER NANOPARTICLES OF HOMOEOPATHIC MEDICINE CURCUMA LONGA – A NANO COMPARATIVE STUDY”

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ABSTRACT.

Background- Growing evidence shows that an active component of turmeric, curcumin, may be used medically to treat a variety of diseases. Nanoparticles have biological activity. Nanoparticles in homeopathic medicines exert biological effects on the body .

Objectives- Homoeopathic mother tincture(Q) of *Curcuma longa* (CL) (Purchased from Willmar Schwabe India Pvt.Ltd) was used for this entire study. In this study our aim was to investigate the phytochemical constituents of *Curcuma longa* Q in their corresponding gold nanoparticle (GNP's) and silver nanoparticle (SNP's) preparations and its comparative analysis.

Methods- *Curcuma longa* Q have been used for the entire study. GNP'S and SNP's were synthesised from Q. 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 homeopathic formulations, synthesised gold and silver nanoparticles.

Results – Phytochemically all the samples have shown the presence of flavonoids. Presence of carbohydrates, steroids, cardiac glycosides and anthraquinone glycosides were detected in both *Curcuma longa* Q and synthesised

gold nanoparticles of *Curcuma longa* Q. Alkaloids and phenols were detected in the samples of *Curcuma longa* Q and SNP preparation of *Curcuma longa* Q . All three samples tested negative for saponins and protein's.

KEYWORDS:

Nanoparticles, Gold nanoparticles, Silver nanoparticles, Homoeopathic medicine, *Curcuma longa*, Phytochemical Assay.

1.INTRODUCTON.

Curcuma longa, is the Homoeopathic medicine prepared from turmeric through the process of potentization. Turmeric (Rhizome)-Southern Asia belongs to the natural order of Zingiberaceae. [5] Turmeric has long been used in Ayurvedic, Homoeopathic and Chinese medicine as an antimicrobial and anti-fungal agent, in the treatment of skin diseases and wound healing [6].

The use of plants for medicines or tonic properties goes back to prehistoric times and has attracted the interest of scientists for centuries. The Vedas give the earliest written record about the science of healing. The reference to medicinal plant is also found in the Ebers papyrus (16th century B.C.) which lists in detail over 7000 herbal remedies [6]

Growing evidence shows that an active component of turmeric, curcumin, may be used medically to treat a variety of dermatological diseases [7]. Nanoparticles have biological activity. Nanoparticles in homeopathic medicines exert biological effects on the body. [2][9]

Reducing particle size increases surface area. Nanoparticles are nano-size particles (1-100nanometres diameter) having a large surface area to volume ratio which gives them different properties from those bulk forms of the same material. [2]

The objective of this study is to understand the phytochemical constituents of *Curcuma longa* Q in their corresponding gold nanoparticle (GNP's) and silver nanoparticle (SNP's) preparations and its comparative analysis.

2 MATERIALS AND METHODS.

SYNTHESIS OF GOLD NANOPARTICLE:

Homoeopathic mother tincture *Curcuma longa* was used for the synthesis of gold nanoparticles .100mg of trichloro gold hydrochloride (chloroauric acid) added to 20 ml of distilled water. Vigorously stirred with 5ml of mother tincture. A change in the colour of the solution was observed.

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 colour of the solution was recorded. (Sana KT et.al.,2021) Similarly, synthesis of other potencies was also done.

CHARACTERISATION OF NANOPARTICLES:

The synthesised nanoparticles were characterized using UV spectroscopy, Scanning electron microscopy (SEM). (Sana KT et.al.,2021) 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 nanoparticles. [8]

PHYTOCHEMICAL ANALYSIS:

Curcuma longa Q ,GNP's and SNP's 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. The ethanol extract was considered suitable since the homoeopathic formulations are in ethanol medium.

Test for flavonoids-Alkaline reagent test :

To different sample solutions, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour, which turned colourless after addition of few drops of dilute hydrochloric acid, indicated the presence of flavonoids.

Test for alkaloids-Mayer's test:

1ml of Mayer's reagent was added to the different sample solutions. Formation of cream colour precipitate indicated the presence of alkaloids.

Test for saponin glycosides :

1ml extract was treated with 1%lead acetate solution. Formation of white precipitate indicates the presence of saponins.

Test for amino acids- Ninhydrin test :

A solution of ninhydrin in ethanol is added to the sample solutions. Appearance of a purple colour indicated the presence of amino acids.

Test for tannins:

Ferric chloride test Different sample solutions were treated with ferric chloride solution; appearance of blue and green colours indicated the presence of hydrolysable and condensed tannins.

Test for carbohydrate :

Benedict's test Plant extracts were treated with benedict's reagent and heated gently. Orange red precipitate indicates the presents of reducing sugar.

Test for steroids:

Salkowski reaction

To the different sample solutions, chloroform was added followed by concentrated sulphuric acid along the sides of the tube. A red-brown colouration indicated the presence of steroids.

Test for cardiac glycosides:

Keller–Killiani test

Glacial acetic acid (0.4 mL) and a few drops of 5% ferric chloride solution are added to the sample solutions. Concentrated sulphuric acid (0.5 mL) is added along the side of the test tube carefully. The formation of blue colour in the acetic acid layer confirmed the presence of cardiac glycosides.

Test for anthraquinone glycosides:

Hydroxyanthraquinone test

To 1 mL of the samples, a few drops of 10% potassium hydroxide solution were added. The formation of a red colour confirmed the presence of anthraquinone glycosides.

Test for proteins:

Biuret test

To 2 mL of the sample solutions, 5 drops of 1% copper sulphate solution are added followed by 2 mL of 10% NaOH. The contents are mixed thoroughly. Formation of a purple or violet colour confirmed the presence of proteins.

3 RESULTS:

Synthesis of gold nanoparticles from homoeopathic mother tincture of *Curcuma longa*.

Synthesis of gold nanoparticles from Homoeopathic Mother tincture of *Curcuma longa*. The Gold nanoparticles were synthesised by addition of trichloro gold hydrochloride (chloroauric acid). This was shown by the change in colour of solution from golden yellow to dark brown. The colour change was observed after 15mins.



(a) Before the reaction.



(b) After the reaction.

Figure 1 : Colour change indicates the formation of gold nanoparticles (GNP'S).

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 2: Colour change indicates the formation of silver nanoparticles (SNP'S).

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.

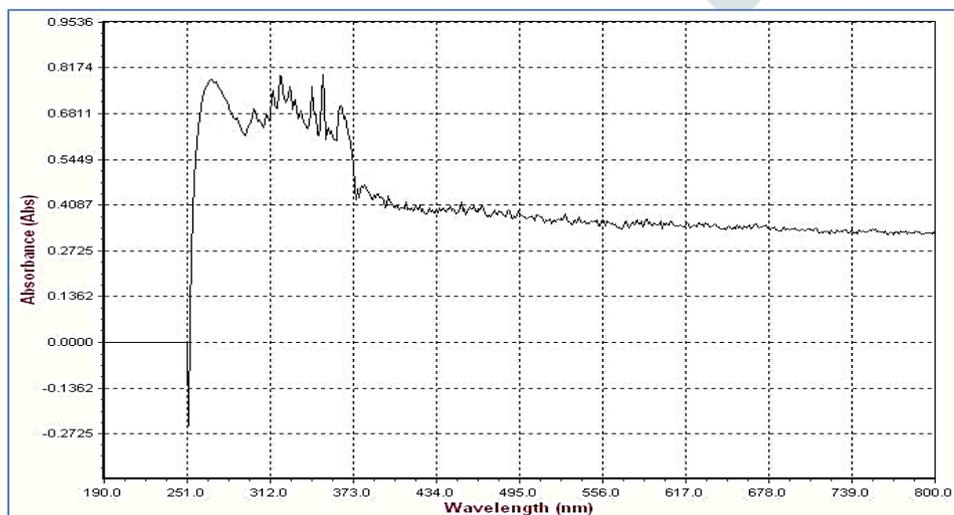
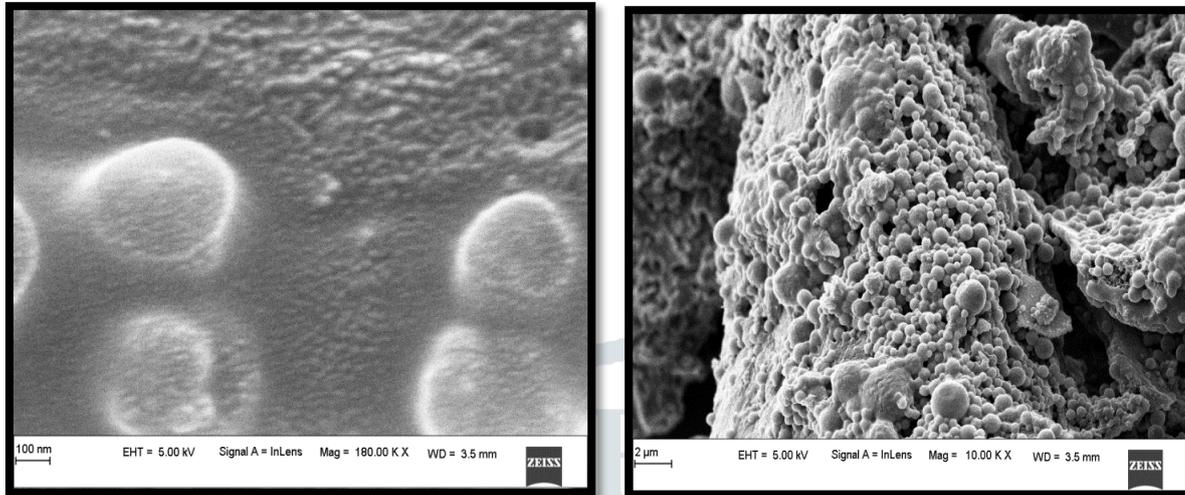
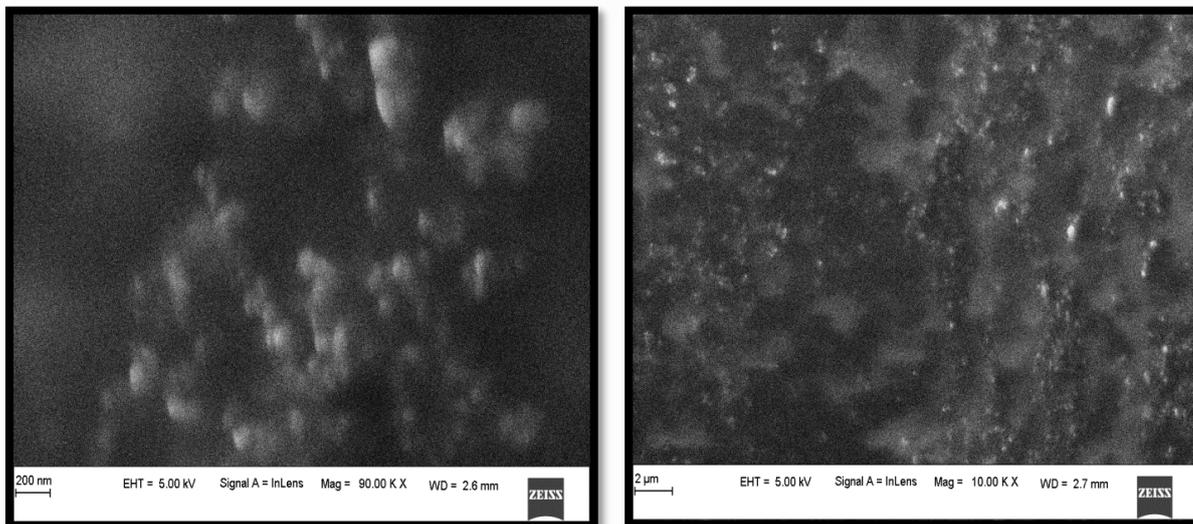


Figure 3: UV-Absorption peak of synthesised SNP's of *Curcuma longa* Q-350 nm.

Scanning Electron Microscopy (SEM):

The size and shape of the particles assessed by SEM is summarized as :**(Fig-4A Mother tincture)** (a)- Spherical particles upto 100nm. **(b)**-Spherical particles upto 2 μ m **(Fig-4B SNP Synthesised Mother tincture)** (c)- Spherical particles upto 200nm. **(d)**-Spherical particles upto 2 μ m.

Figure 4A: Mother tincture**(Figure-a)****(Figure-b)****Figure 4B: Synthesised Mother tincture****(Figure-c)****(Figure-d)****Figure 4-Results of Scanning Electron microscopy (SEM)**

3.3 PHYTOCHEMICAL ANALYSIS

NO	PHYTOCHEMICAL CONSTITUENTS	CL Q	GNP Q	SNP Q
1	FLAVONOIDS.	+	+	+
2	ALKALOIDS.	+	-	+
3	SAPONINS	-	-	-
4	PHENOLS	+	-	+
5	TANNINS.	-	-	-
6	CARBOHYDRATES.	+	+	-
7	STEROIDS.	+	+	+
8	CARDIAC GLYCOSIDES.	+	+	-
9	ANTHRAQUINONE GLYCOSIDES.	+	+	-
10	PROTEINS.	-	-	-

Table 1: *Phytochemical Analysis Result-Curcuma longa Q, synthesised GNP's AND SNP'S*

Phytochemically all the samples have shown the presence of flavonoids. Presence of carbohydrates, steroids, cardiac glycosides and anthraquinone glycosides were detected in both *Curcuma longa* Q and synthesised gold nanoparticles of *Curcuma longa* Q. Alkaloids and phenols were detected in the samples of *Curcuma longa* Q and SNP preparation of *Curcuma longa* Q. All three samples tested negative for saponins and protiens. These bio active compounds exerts therapeutic effect in the cure of wide range of diseases and the nanoparticles in homeopathic medicines exert biological effects on the body.

4.DISCUSSION:

Nanoparticles in homeopathic medicines exert biological effects on the body. Nanoparticles can cause hormesis. By UV spectrometer peaks we had concluded that reduction has taken place in the solution. The colour change in the reaction mixture was analysed by visual observation. Using SEM, the size and shape of particle in nanometre has been obtained. The detailed characterization was conducted in our previous study (Sana KT et.al.,2021), by which we concluded that homeopathic medicine *Curcuma longa* and its biosynthesised silver nanoparticles are potent anti-oxidant and anti-microbial agents.[8] Phytochemically all the samples have shown the presence of flavonoids. Presence of carbohydrates, steroids, cardiac glycosides and anthraquinone glycosides were detected in both *Curcuma longa* Q and synthesised gold nanoparticles of *Curcuma longa* Q. Alkaloids and phenols were detected in the samples of *Curcuma longa* Q and SNP preparation of *Curcuma longa* Q. All three samples tested negative for saponins and proteins. As we found this plant-based medicine in homeopathy *Curcuma longa* is a vital phytochemically active medicine and this study widens its effectiveness and scope in the the treatment of wide range of diseases.

5.CONCLUSION:

From our results it was evident homeopathic medicines and the synthesised GNP'S and SNP's were found to be phytochemically active and phytochemically all the samples have shown the presence of flavonoids. Presence of carbohydrates, steroids, cardiac glycosides and anthraquinone glycosides were detected in both *Curcuma longa* Q and synthesised gold nanoparticles of *Curcuma longa* Q. Alkaloids and phenols were detected in the samples of *Curcuma longa* Q and SNP preparation of *Curcuma longa* Q. All three samples tested negative for saponins and proteins.

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REFERENCES:

- [1] Bell, I.R. and Schwartz, G.E., 2013. Adaptive network nanomedicine: an integrated model for homeopathic medicine. *surfaces*, 83, p.84.
- [2] Malik, N., 2015. Recent Advances in Nanoparticle Research in Homeopathy. *Recent Advances in Nanoparticle Research in Homeopathy (June 18, 2015). Homeopathy for Everyone*, 12(6).
- [3] Momtaz, S., Lall, N. and Basson, A., 2008. Inhibitory activities of mushroom tyrosine and DOPA oxidation by plant extracts. *South African Journal of Botany*, 74(4), pp.577-582.
- [4] Majeed, M. and Prakash, L., 2008. A Lighter Skin Tone and More... With Natural Actives.
- [5] Akram, M., Shahab-Uddin, A.A., Usmanghani, K.H.A.N., Hannan, A.B.D.U.L., Mohiuddin, E. and Asif, M., 2010. *Curcuma longa* and curcumin: a review article. *Rom J Biol Plant Biol*, 55(2), pp.65-70.
- [6] Krup, V., Prakash, L.H. and Harini, A., 2013. Pharmacological activities of turmeric (*Curcuma longa* Linn): a review. *J Homeop Ayurv Med*, 2(133), pp.2167-1206.

- [7] Vaughn, A.R., Branum, A. and Sivamani, R.K., 2016. Effects of turmeric (*Curcuma longa*) on skin health: a systematic review of the clinical evidence. *Phytotherapy Research*, 30(8), pp.1243-1264.
- [8] K.T. Sana, E. P Jenitta, C. Ingole.Archana., Dhivya.Maria, 2021.Homoeopathy in Nano-particulate perspective -synthesis of silver nanoparticle and effect of homoeopathic medicine curcuma longa including its anti-oxidant and antimicrobial activity. Volume 10, Issue 1.
- [9] Upadhyay, R.P. and Nayak, C., 2011. Homeopathy emerging as nanomedicine. *International Journal of High Dilution Research-ISSN 1982-6206*, 10(37), pp.299-310.
- [10] Wu, L., Chen, C., Cheng, C., Dai, H., Ai, Y., Lin, C. and Chung, Y., 2018. Evaluation of tyrosinase inhibitory, antioxidant, antimicrobial, and antiaging activities of *Magnolia officinalis* extracts after *Aspergillus niger* fermentation. *BioMed research international*, 2018.
- [11] Shah, R., 2016. Standardization of the potentizing machine and quantification of impact of potentization. *Indian Journal of Research in Homoeopathy*, 10(2), p.126.
- [12] Jain, S., Shrivastava, S., Nayak, S. and Sumbhate, S., 2007. Recent trends in *Curcuma longa* Linn. *Pharmacognosy Reviews*, 1(1).
- [13] Murphy robin. Lotus materia medica 1400 homoeopathic and herbal remedies; third edition. B.jain publishers(p) ltd
- [14] Rajendran ES. Nanodynamics – Nanoscience, Homeopathy, Physical sciences, Nanomedicine. 1st ed. Cochin: Mohna Publications; 2015
- [15]Pratim, M.P. and Biman, M., 1999. Text book of homoeopathic pharmacy.
- [16]. Wikipedia (www.wikipedia.com).