

STUDY OF HOMOEOPATHIC MEDICINE CURCUMA LONGA AND ITS BIOSYNTHESED SILVER NANOPARTICLES – ANTI FUNGAL AND ANTI MELANIN ACTIVITY – A NANO-COSMETOLOGICAL PERSPECTIVE

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ABSTRACT: Background- Nanoscale science (or nanoscience) studies the phenomena, properties and responses of materials at atomic, molecular and macromolecular scales, and in general at sizes between 1-100 nm. Recent studies reveal that homeopathic remedies contain nanoparticles (NP's) of source materials formed by potentization. Skin is an important component of body image and has immense physiological importance for both women and men. Skin pigmentation can be a source of significant emotional distress in individuals. Along human history, people have been struggling with numerous skin diseases, especially skin pigmentation (hyper/hypo-pigmentation). It is well documented that tyrosinase is an essential enzyme, which contributes towards pigment formation in mammalian's body as well as in plants, microorganisms and fungi. The rate-limiting step in melanogenesis is the conversion of L-DOPA to melanin, through the action of tyrosinase enzyme. Area of interest of this study focus on this step of melanogenesis.

Objectives- Homoeopathic mother tincture(Q) and potencies(dilutions) 6CH, 12CH, 30CH, 200CH & 1M 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 anti-fungal and anti-melanin activity (mushroom tyrosinase inhibitory activity) of *Curcuma longa* Q, potencies and their corresponding silver nanoparticle (SNP's) preparations and its application in a Cosmetological perspective.

Methods- SNP's were synthesised from Q, potencies and characterisation was done using UV-spectroscopy and Scanning Electron Microscopy (SEM). Its anti-fungal activity and anti-melanin activity (mushroom tyrosinase inhibitory activity) was studied in detail using Agar well diffusion method and Mushroom tyrosinase inhibitory assay by using L-DOPA respectively.

Results - 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 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 up to 100nm. The antifungal activity for *Aspergillus niger* shows highest zone of inhibition of 10mm for Q and 1M. And for *White rot fungi* highest zone of inhibition of 10mm was obtained for CL 1M SYN. The mushroom tyrosinase inhibitory assay shows highest inhibition rate of 87.92±.041% for CL 30C (IC50 83.33mg) and lowest inhibitory activity of 56.67±.046% for CL 200C (IC50 138.8±24mg). The medicinal and SNP synthesised samples of CL was showing a very potent mushroom tyrosinase inhibitory activity.

Conclusions- Through this study it was evident that homoeopathic medicines and the synthesised SNP'S were found to be effective as an anti-fungal agent against *Aspergillus niger* and *White rot fungi* which are causative of many fungal infections. As well as it was found to be a potent tyrosinase inhibitor in mushroom tyrosinase assay. Hence, it can be used as an anti-melanin agent in treating various skin pigmentations and Cosmetological aspects.

KEYWORDS:

Nanoparticles, Silver nanoparticles, Homoeopathic medicine, Potentization, *Curcuma longa*, Antifungal activity, Anti melanin activity, Mushroom tyrosinase inhibition assay,

1.INTRODUCTON.

Homeopaths treat disease using very low dose preparations administered according to the principle that "like should be cured with like". Homeopathic medicines are prepared by a pharmacological process of potentization/trituration. Recent studies reveal that homeopathic remedies contain nanoparticles (NPs) of source materials formed by potentization [1]. This results in breaking down of medicinal substance into nanoparticles. Homeopathy is in fact, a form of Nano-pharmacology. 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]

Along human history, people have been struggling with numerous skin diseases, especially skin pigmentation (hyper/hypo-pigmentation). It is well documented that tyrosinase is an essential enzyme, which contributes towards pigment formation in mammalian's body as well as in plants, microorganisms and fungi. Use of tyrosinase inhibitors is becoming increasingly important in the cosmetic industry due to their skin-whitening effects.

Melanin plays an important role in protecting human skin from the harmful effects of ultraviolet (UV) radiations by absorbing UV sunlight, removing reactive oxygen species (ROS) and scavenging toxic drugs and chemicals. The accumulation of an abnormal melanin amount in specific parts of the skin as more pigmented patches (melasma, freckles, ephelide, senile lentiginos etc.) might become an aesthetic problem. The type and amount of melanin synthesized by the melanocytes and its distribution in the surrounding keratinocytes determine the actual colour of the skin [3]

Melanin biosynthesis (melanogenesis) is influenced by genetics, environmental factors, diet and medication. The production of melanin by specialized cells called melanocytes (in the basal layer of the epidermis in light skinned people and in the basal as well as horny layer in dark skinned people) occurs through the action of the enzyme tyrosinase. The rate-limiting step in melanogenesis is the conversion of L-DOPA to melanin, through the action of tyrosinase enzyme. Tyrosinase is a multicopper monooxygenase enzyme with wide distribution [4].

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 anti-fungal agent, in the treatment of skin diseases and wound healing [6].

Growing evidence shows that an active component of turmeric, curcumin, may be used medically to treat a variety of dermatological diseases and has got anti-melanin activity [7]. This systematic review led to examine the evidence for the use of homoeopathic medicines of turmeric/curcumin (*Curcuma longa*) to modulate the melanogenesis and its effectiveness as a tyrosinase inhibitor (anti-melanin agent) in skin pigmentations.

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 anti-fungal activity and anti-melanin activity (mushroom tyrosinase inhibitory activity) and its application in a Nano-Cosmetological perspective.

2.MATERIALS AND METHODS.

2.1 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.

2.2 CHARACTERISATION OF SILVER NANOPARTICLES:

The synthesised SNP's 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 silver nanoparticles. [8]

2.3 ANTI-FUNGAL ACTIVITY:

Preparation of the fungal cultures and isolation of *Aspergillus niger* and *White rot fungi* were done at Alva's Centre for Research in Nanotechnology.

Petri dishes were plated with Potato dextrose Agar (of Himedia Laboratories Pvt. Ltd, purchased from Durga Laboratories Mangalore) media and allowed to solidify for 30 minutes. The organism was spread on surface of the media using sterile swab stick. Cork borer (7mm) was used to bore wells in media .40µl of medicinal and SNP'S extract in different potencies (6CH, 12CH,30CH, 200CH,1M and Q) were dispensed into the wells using a micropipette. A positive control of fluconazole 30mcg/disc was kept and the extract was allowed to diffuse for 1 hr at room temperature. Then the plates were kept at 37°C for 7 days. Zones of inhibition in mm were measured.

2.4 ANTI-MELANIN ACTIVITY:

MUSHROOM TYROSINASE INHIBITION ASSAY:

Tyrosinase inhibition assays were performed with LDOPA as substrate. The reaction mixture contained 3.425ml of phosphate buffer (0.05 M, pH 6.5), .075ml of mushroom tyrosinase (2500 U mL⁻¹) (purchased from SISCO Research Laboratories Pvt.Ltd), 1ml of plant extract solution and 500µg of 5 mM L-DOPA (purchased from Durga Laboratories, Mangalore). After the addition of L-DOPA, the reaction was immediately monitored at 490 nm for dopachrome formation in the reaction mixture. The concentration range of extract used for the mushroom tyrosinase inhibition assay was .25–2 mg/mL.

Anti – tyrosinase activity (%) = $\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$

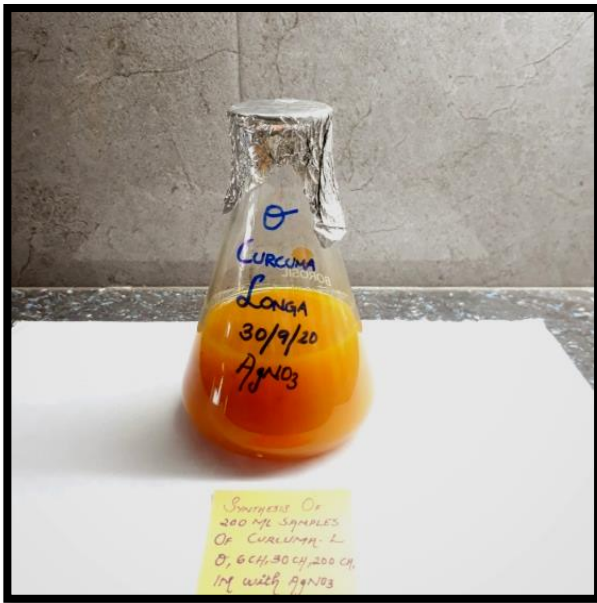
Where, Abs control is absorbance of control at 490 nm, Abs sample is absorbance of sample at 490 nm. The IC₅₀ value, a concentration giving 50 % inhibition of tyrosinase activity, was determined by interpolation of concentration-response curves. Kojic acid (purchased from Durga Laboratories, Mangalore) was used as the reference tyrosinase inhibitor. All tests were performed in triplicate.

The anti-tyrosinase activity for *Curcuma longa* (Q), potencies and corresponding SNP synthesised samples was determined for its inhibition mechanism by using a Lineweaver–Burk plot standard.

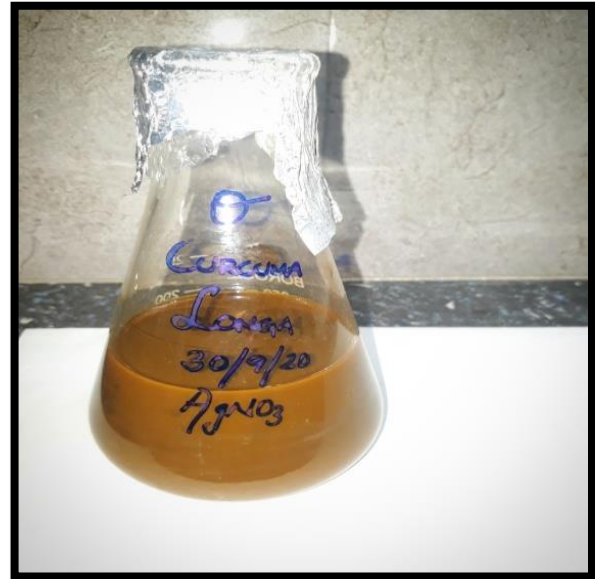
3. RESULT:

3.1 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 silver nanoparticles.

3.2 Characterisation of Silver Nanoparticles:

3.2.1 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.

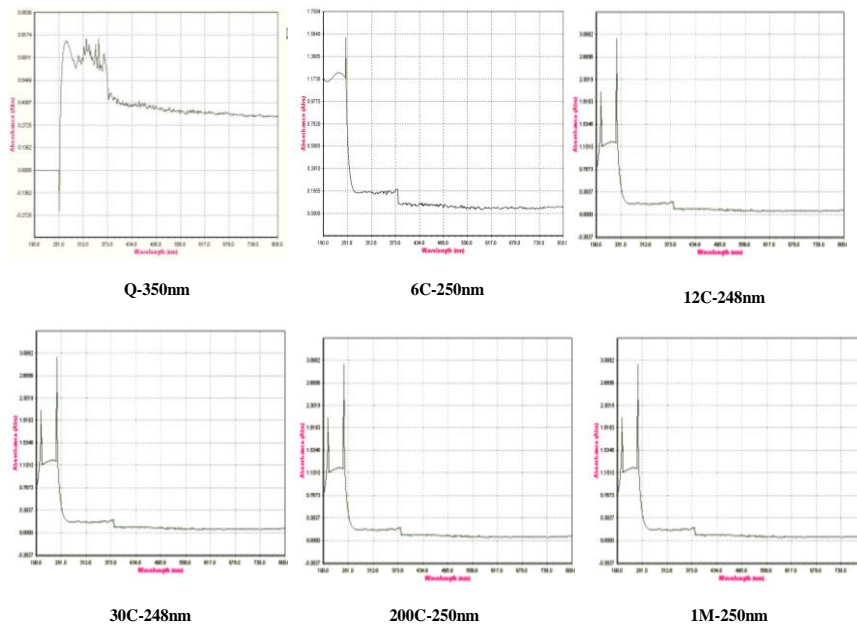
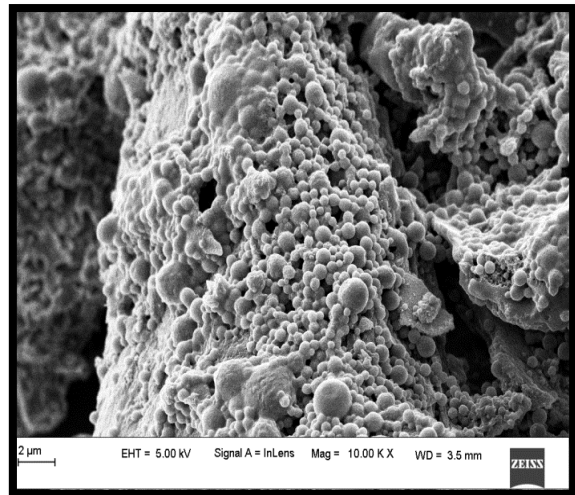
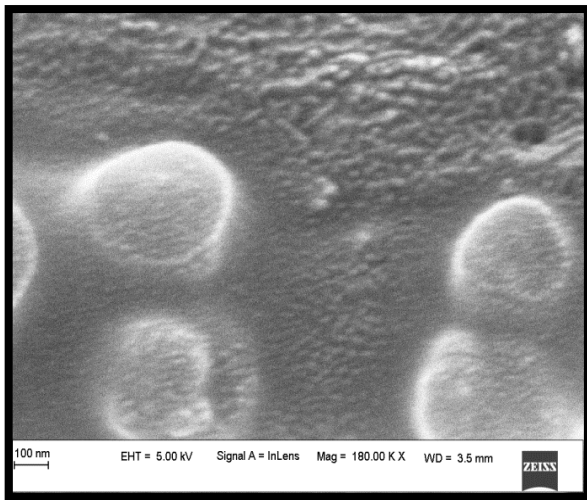


Figure 2: UV-Absorption peak of synthesised SNP's of *Curcuma longa*.

3.2.2 Scanning Electron Microscopy (SEM):

The size and shape of the particles assessed by SEM is summarized as : (Fig-3.1 Mother tincture) (3.1.1)- Spherical particles upto 100nm. (3.1.2)-Spherical particles upto 2µm (Fig-3.2 Synthesised Mother tincture) (3.2.1)- Spherical particles upto 200nm. (3.2.2)-Spherical particles upto 2µm. (Fig-3.3 Synthesised 6C Potency) (3.3.1)- Spherical particles upto 100nm. (3.3.2)-Spherical particles upto 2µm.

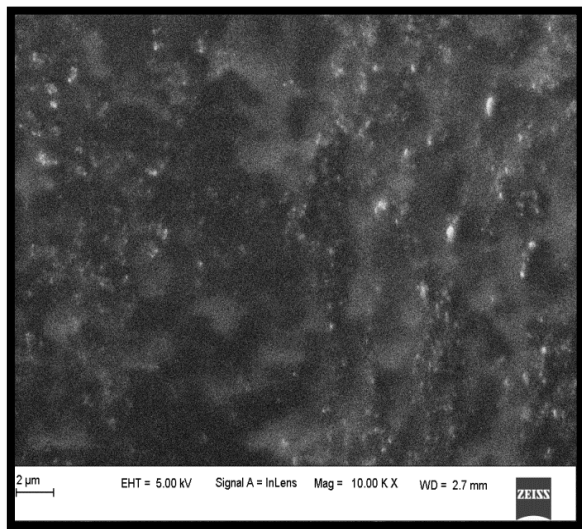
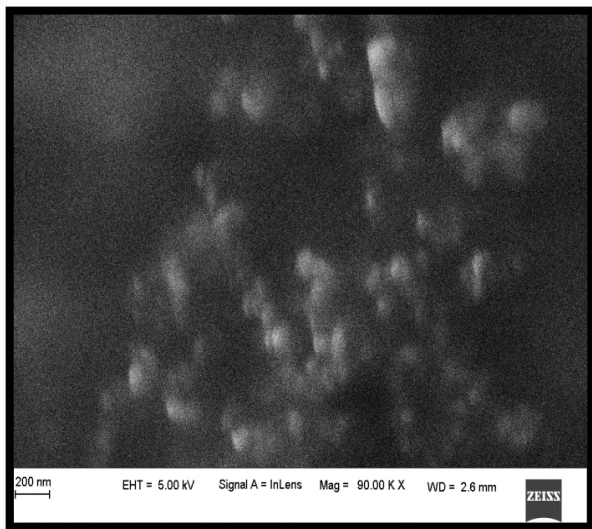
Figure 3.1: Mother tincture



(Figure-3.1.1)

(Figure-3.1.2)

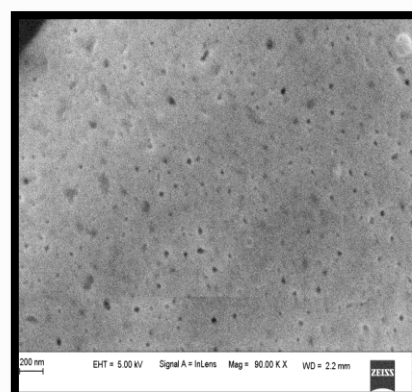
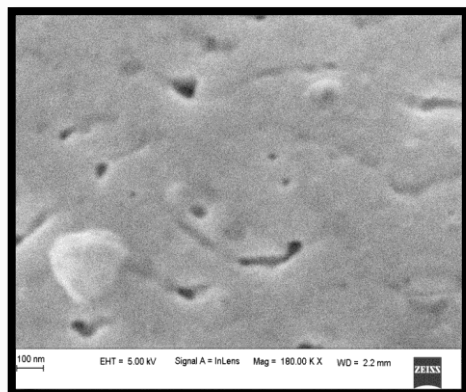
Figure 3.2: Synthesised Mother tincture



(Figure-3.2.1)

(Figure-3.2.2)

Figure 3.3: Synthesised 6C Potency:



(Figure-3.3.1)

(Figure-3.3.2)

Figure 3-Results of Scanning Electron microscopy (SEM)

3.3 Anti-fungal Activity:

The anti-fungal activity was done by agar well diffusion method. The antifungal activity for *Aspergillus niger* shows highest zone of inhibition of 10mm for CL Q and CL 1M , 9mm for CL12C and CL30C,8mm for CL6C , CL 1M SYN,7mm for CL Q SYN,CL 12C SYN,CL 30C SYN ,CL 200C SYN followed by 6mm for CL 200C and CL 6C SYN.

And for *White rot fungi* species highest zone of inhibition of 10mm was obtained for CL 1M SYN 8mm for CL Q SYN, CL 1M,7mm for CL Q, CL6C SYN, CL12C SYN, CL 30C SYN, CL 200C SYN,6mm for CL 6C, CL 12C, CL 30C, CL 200C.

Zone of inhibition in mm(40μl)

SL .NO	MICRO- ORGANISM	CLQ	CL 6C	CL 12C	CL 30C	CL 200C	CL 1M
1.	<i>Aspergillus niger</i>	10	8	9	9	6	10
2.	<i>White rot fungi</i>	7	6	6	6	6	8
3.	<i>Fluconazole (control)</i>	5	5	5	5	5	5

Table 1: Anti-fungal activity with Homeopathic medicine.

SL .NO	MICRO- ORGANISM	CLQ	CL 6C	CL 12C	CL 30C	CL 200C	CL 1M
1.	<i>Aspergillus niger</i>	7	6	7	7	7	8
2.	<i>White rot fungi</i>	8	7	7	7	7	10
3.	<i>Fluconazole (control)</i>	5	5	5	5	5	5

Table 2: Antifungal activity with synthesised silver nanoparticles in Homoeopathic medicine.

3.4 ANTI-MELANIN ACTIVITY

(MUSHROOM TYROSINASE INHIBITION ASSAY):

Table 3: Anti- tyrosinase inhibitory activity with Homeopathic medicine.

CONCENTRATION (mcg)	250	500	1000	2000		
	% of inhibition 1	% of inhibition 2	% of inhibition 3	% of inhibition 4	IC50 (mg)	MEAN % OF INHIBITION
Q	91.67±.02	81.67±.02	75.00±.05	65.00±.05	100±.00	78.33±.039
12C	85±.05	81.67±.028	76.67±.028	75±.05	94.44±9.62	79.58±.039
6C	71.67±.028	61.67±.028	55.00±.05	45±.05	138.8±24	58.33±.039
30C	93.33±.028	91.67±.028	85±.05	81.67±.057	83.33	87.92±.041
200C	66.67±.028	58.33±.028	55.00±.05	46.67±.076	138.8±24	56.67±.046
1M	88.33±.028	80.00±.086	68.33±.076	56.67±.160	108.3±14,	73.33±.088

Figure 4: Lineweaver-Burk plots of Mushroom tyrosinase and L DOPA in the presence of *Curcuma longa* Q,6C,12C,30C,200C,1M medicinal samples.

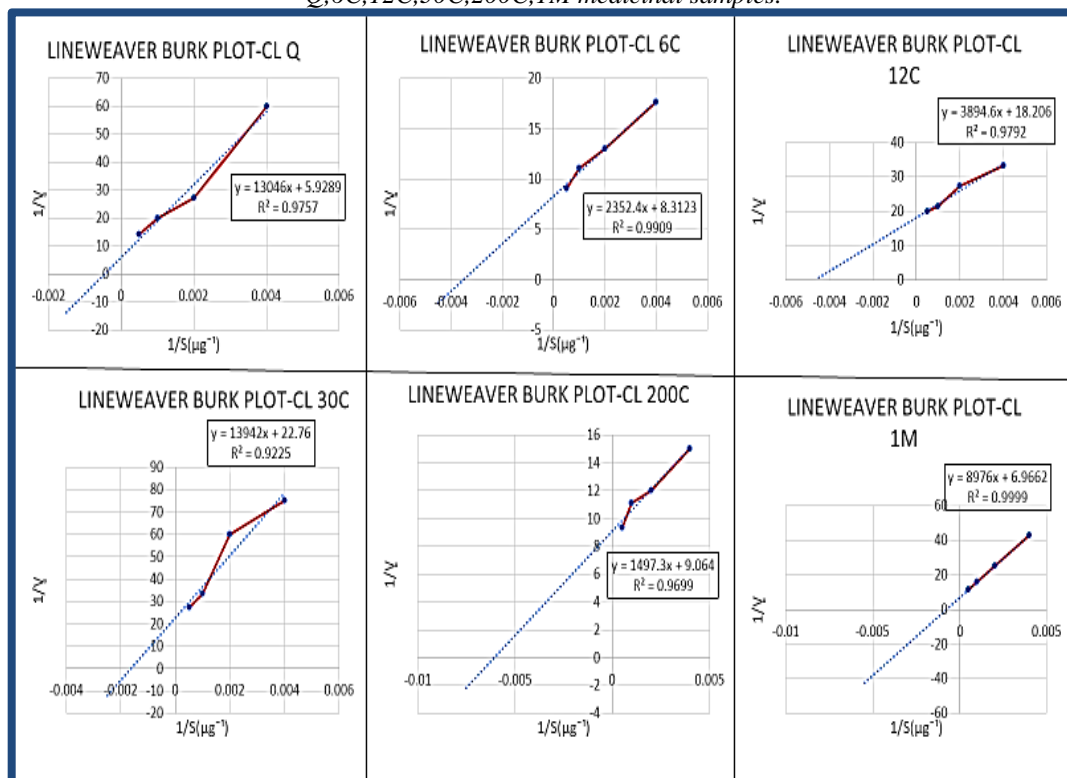


Figure 5: Concentration-Response curves of *Curcuma longa* Q,6C,12C,30C,200C,1M medicinal samples.

CONCENTRATION(mcg)	250	500	1000	2000	IC50	
	% of inhibition 1	% of inhibition 2	% of inhibition 3	% of inhibition 4	IC50 (mg)	MEAN % OF INHIBITION
QSYN	86.67±.028	76.67±.028	73.33±.028	65±.028	100	75.42±.034
6CSYN	91.67±.028	86.67±.028	73.33±.057	65±.05	100±.00	79.17±.04
12CSYN	90±.05	88.33±.028	80±.05	78.33±.076	88.88±9.62	84.17±.05
30C SYN	71.67±.028	61.67±.028	55.00±.05	45±.05	138.8±24	71.25±.06
200 SYN	83.33±.028	81.67±.028	75.00±.05	73.33±.028	100.00±0mg	78.33±.034
1MSYN	86.67±.076	80.00±.05	83.33±.076	78.33±.07	88.8±9.6	82.08±.069

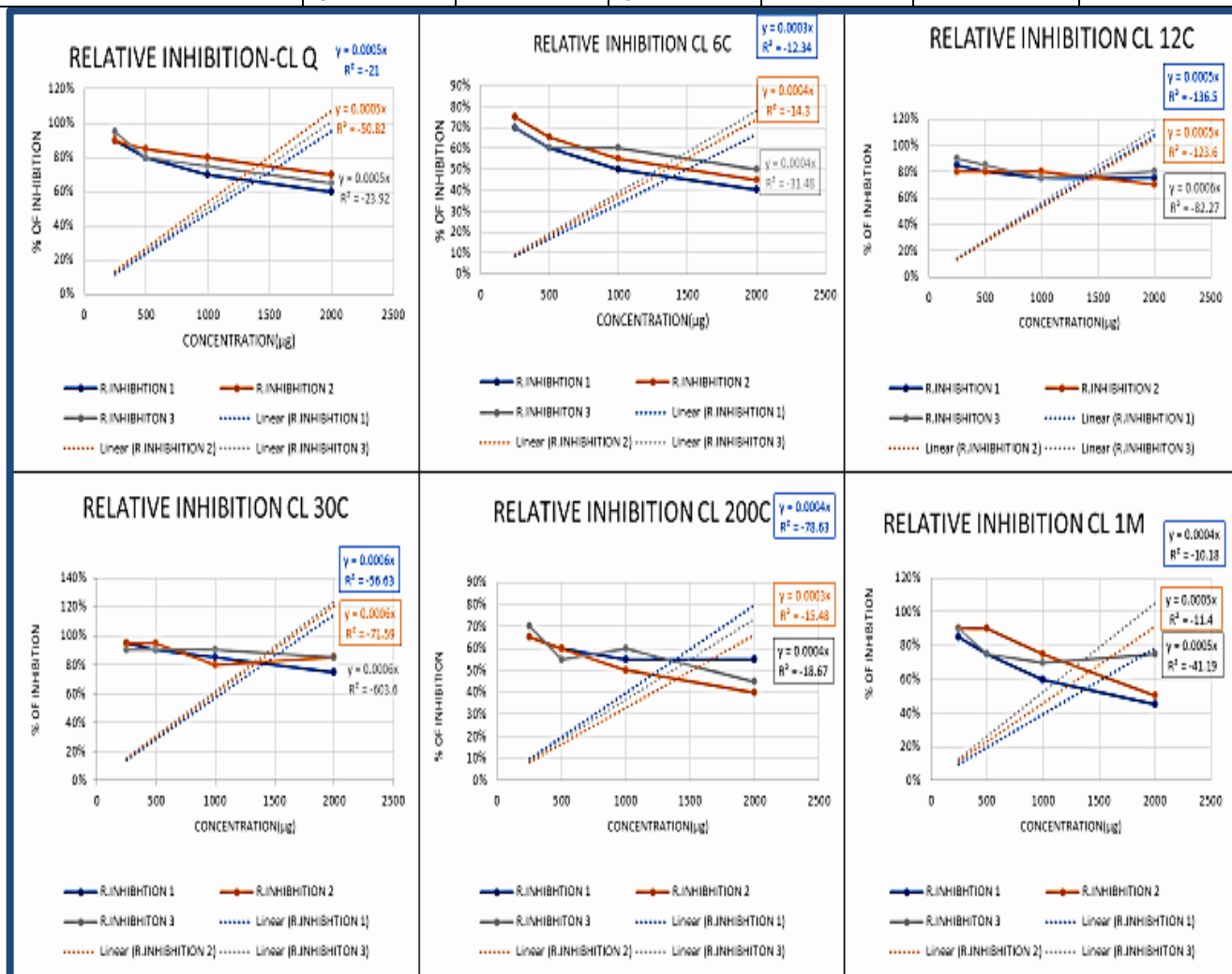


Table 4: Anti tyrosinase inhibitory activity with synthesised silver nanoparticles in Homeopathic medicine.

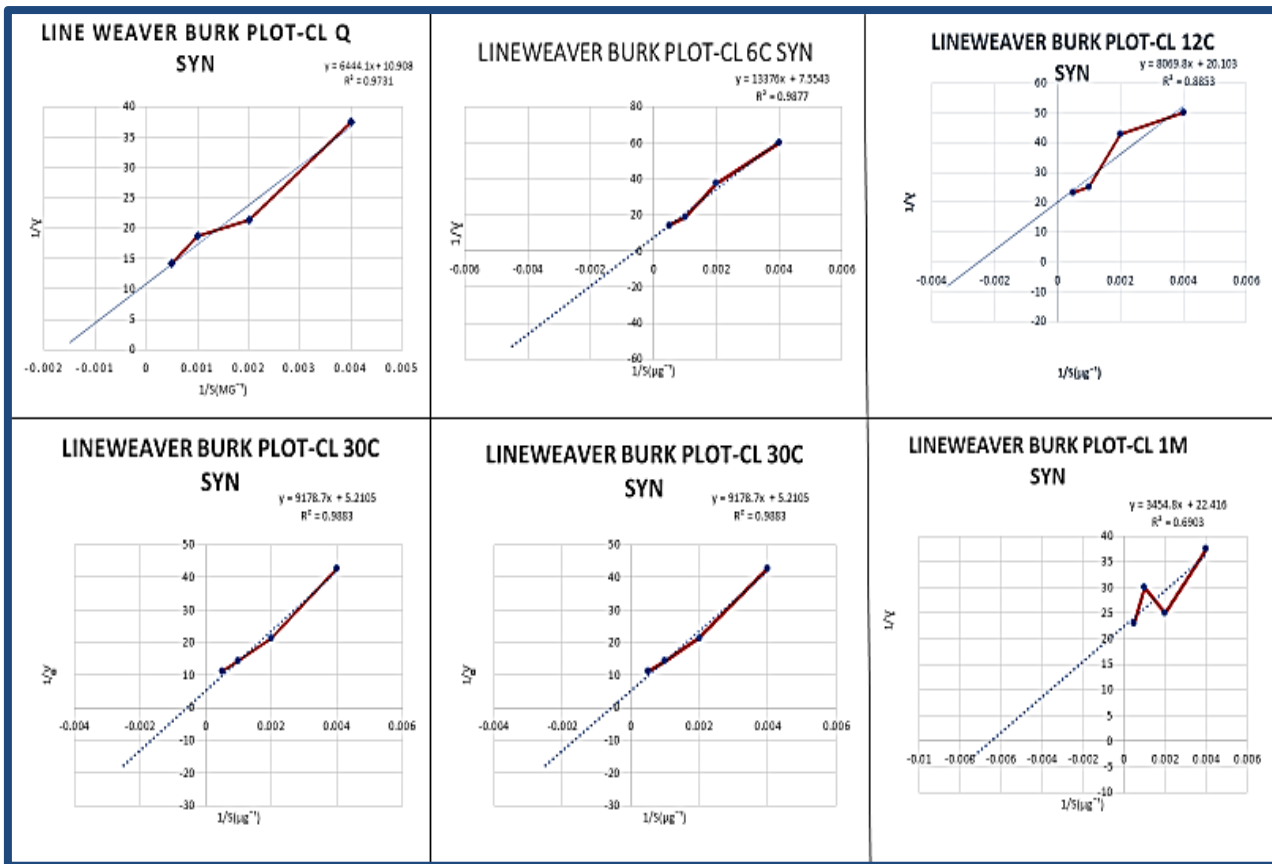


Figure 6: Lineweaver-Burk plots of Mushroom tyrosinase and L DOPA in the presence of SNP'S synthesised Curcuma longa Q,6C,12C,30C,200C,1M samples.

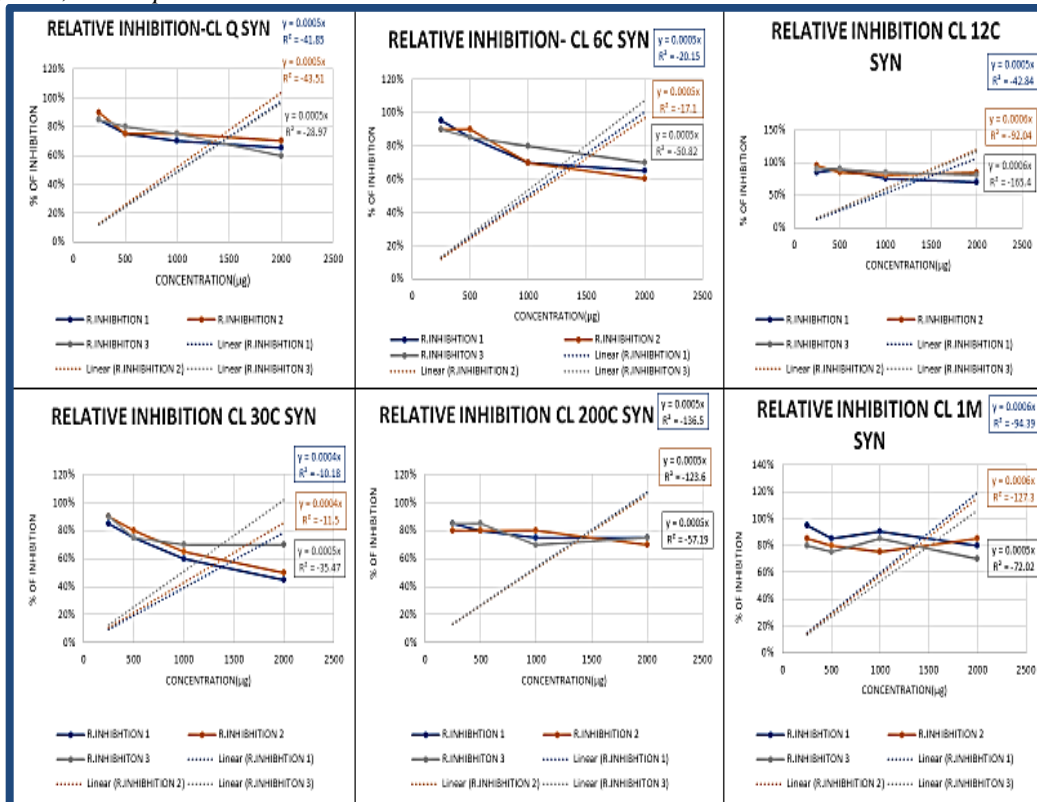


Figure 7: Concentration-Response curves of SNP'S synthesised Curcuma longa Q,6C,12C,30C,200C,1M samples.

4.DISCUSSION:

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. Hormesis is akin to Arndt Schultz Law which is about nonlinearity between dose and response. Hence, it appears that homeopathically prepared remedies may also stimulate a similar response, causing a specific hormesis which, in turn, ensures that homeostasis is maintained, and thus, counteracts illness in a curative manner. [9] 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]By this study using homeopathic medicine *Curcuma longa* the Anti-fungal action of homeopathic medicine *Curcuma longa* were studied on organisms *Aspergillus niger* and *White rot fungi* and a positive result with transparent inhibition circumference around the wells has been observed, which confirmed that the fungi were unable to grow in and around the medicinal and SNP's synthesised samples. The mushroom tyrosinase inhibition assay (anti-melanin activity) has proved *Curcuma longa* (medicine and SNP's synthesised samples) are potent tyrosinase inhibitors (anti-melanin agents), with highest inhibition rate of $87.92 \pm 0.041\%$ for CL 30C potency. Nontoxic natural products used in formulating cosmetics and pharmaceuticals are of considerable interest. Natural products made from plant sources have been used in cosmetic applications as whitening agents and as a nutritional source [10]. As we found this plant-based medicine in homeopathy *Curcuma longa* is a vital tyrosinase inhibitor, this study widens its effectiveness and scope in the Cosmetological and dermatological conditions like pigmented patches, melasma, freckles, ephelide, senile lentigines, post inflammatory hyperpigmentation's, hyper pigmented scars, acne etc.

5.CONCLUSION:

From our results it was evident homeopathic medicines and the synthesised SNP's were found to be effective as an anti-fungal agent against *Aspergillus niger* and *White rot fungi* which are causative of many fungal infections. As well as it was found to be a potent tyrosinase inhibitor in mushroom tyrosinase assay. Hence, it can be used as an anti-melanin agent as an external applicant in treating various skin pigmentations and Cosmetological aspects.

ACKNOWLEDGEMENTS:

We would like to thank our Alva's Education Foundation, Moodbidri, Mangalore for the immense support in the field of research and infrastructure.

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