ANTI-CANCER PROPERTIES OF BIOSYNTHESIZED SILVER AND GOLD NANOPARTICLES FROM HOMOEOPATHIC DRUG CONDURANGO MOTHER TINCTURE AND ITS HOMOEOPATHIC ULTRA DILUTIONS-AN IN-VITRO STUDY

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Abstract: As the advancement of nanoscience, synthesis of silver (AgNPs) and gold nanoparticle (AuNPs) using plant extract and its scope in the treatment of varieties of cancers has got a special attention in medical field now a days. Condurango is a Homoeopathic medicine derived from plant kingdom, which is commonly used for the treatment of mouth, esophageal and stomach cancer. In this study Condurango (CON) Q and its various Homoeopathic potencies such as CON 6C, CON 12C, CON 30C, CON 200C, CON 1M, CON 10M, CON 50M, CON CM were used for biosynthesis of AgNPs and AuNPs. Characterization of Synthesized AgNPs and AuNPs along with Homoeopathic medicine CON Q to CON CM potencies were done using ultraviolet-visible (UV)-spectroscopy, FTIR, SEM, & TEM analysis. Phytochemical analysis, Antioxidant properties using DPPH Assay and Antimicrobial Activity of CON Q to CM potency and Synthesized AgNPs and AuNPs also carried out. To study the anti-cancerous property cell viability test was done on KB-3-1 Cell line using MTT assay. The results revealed AgNPs and AuNPs were synthesized using CON Q to CON CM potencies and biosynthesized AgNPs, AuNPs and CON Q to CON CM potencies were having significant antioxidant, antimicrobial and anti-cancerous properties.

Keywords: Biosynthesis, Homoeopathic Medicines, Nanoparticles, Condurango, Oral cancer, Silver nanoparticle, Gold nanoparticles

I. INTRODUCTION

Oral cancer (cancer of lips, mouth and tongue) is an unavoidable health challenge in developing countries like India [1]. Among different types of cancers, oral cancer ranks 6th in the world [2]. Recent statistics shows prevalence of oral cancer in India annually is around 77,000 new cases and 52,000 deaths (nearly one fourth of global prevalence) [3]. Oral squamous cell carcinoma contributes 84-97% of oral cancer. OSCC is one of the potentially malign lesions of oral cavity. Preclinical phases of oral cancer such as inflammatory oral submucosa, fibrosis, erythroplakia, leukoplakia, candida leukoplakia, dyskeratosis congenital, and lichen planus need to be attended seriously [4]. High prevalence of oral cancer in India contributes to a number of etiological factors. Tobacco or smoking, alcohol addiction is the common cause of oral cancer [5]. This forms the serious health hazard in low- and middle-class socio-economic status of Indian population. Hence finding the safer and effective treatment for oral cancer becomes crucial. Advancement of nanotechnology and the properties of nanoparticles such as physiochemical and biological properties, have important role in drug delivery, sensors, optoelectronics, and magnetic devices. So, this study focuses on the bio synthesis of silver nanoparticles (AgNPs) and gold nanoparticles using Homoeopathic medicine CON Q and its ultra-dilutions. Bio- synthesis of nanoparticles using Plant extracts is an eco-friendly approach [6]. The synthesis AgNPs and AuNPs using plant extract had made a significant change in the field of medicine for its medical use. It is proved that biosynthesized AgNPs and AuNPs have great potential to act as antimicrobial, antioxidant, and anticancer activities [7]. The recent discovery of nanoparticles in traditional Homoeopathic medicines added another point of convergence between modern nanomedicines and alternative interventional strategies [8]. Homoeopathic drug Condurango (CON) is the one of the commonest medicines used as a palliative curative remedy for cancer of mouth & stomach [9]. So, this study was undertaken to see the effects CON and it's all potencies, with biosynthesized Silver (AgNPs) and Gold (AuNPs) nanoparticles using CON Q, 6, 12, 30, 200, 1M, 10M, 50M, CM for the treatment of oral cancers and to standardize a nano Homoeopathic medicine for the treatment of oral cancer.

II. MATERIAL & METHODS

2.1. Collection of Materials

Mother tincture and Potencies (CON 6, 12, 30,200, 1M, 10M, 50M & CM) of CON drug for research was procured from GMP certified Homoeopathic Pharmaceutical industry.

2.2. Phytochemical Analysis

CON Q, CON 6C, CON 12C, CON 30C, CON 200C, CON 1M, CON 10M, CON 50M & CON CM was used for all the experiment in this study. The reagents used for phytochemical analysis have been prepared following standard protocol followed in Alva's nanotechnology lab.

2.2.1 Test for Amino Acids (Ninhydrin test):

Ninhydrin Solution in ethanol is added drop wise to the 5ml of sample solutions. Change in colour of sample solution into purple colour indicated the presence of amino acids.

2.2.2 Test for Tannins (Ferric chloride test):

All the sample solutions were treated with ferric chloride solution; appearance of blue and green colour indicated the presence of hydrolysable and condensed tannins.

2.2.3 Test for Carbohydrate (Benedict's test):

Samples were treated with benedict's reagent and heated gently. Colour changes were observed. Orange to red precipitate indicates presence of carbohydrates.

2.2.4 Test for Steroids-Salkowski Reaction:

To the sample solutions, few drops of chloroform was added followed by concentrated sulphuric acid along the sides of the test tube. A red-brown colour change indicated the presence of steroids.

2.2.5 Test for Cardiac Glycosides-Keller-Killiani Test:

Few drops of Glacial acetic acid (0.4 mL) and 5% ferric chloride solution were added to the sample solutions. Then concentrated sulphuric acid (0.5 mL) was added along the side of the test tube carefully. The formation of blue colour layer confirmed the presence of cardiac glycosides.

2.2.6 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.

2.2.7 Test for Proteins-Biuret Test:

To 5 mL of the sample solutions, 10 drops of 1% copper sulphate solution was added followed by 2 mL of 10% NaOH. Then contents were mixed thoroughly. Change of colour of the solution to purple or violet colour confirmed the presence of proteins 2.3 Synthesis of Silver Nanoparticle (AgNPs):

Homoeopathic Drug CON Q to CM potencies were used for the synthesis of AgNPs. 0.1gm of silver nitrate (AgNO3) was added

to 100 ml of distilled water and then 20 ml of CON Q was added and vigorously stirred. A change in the colour of solution was observed. Similarly, synthesis of AgNPs was done using other potencies such as CON 6, CON 12, CON 30, CON 200, CON 1M, CON 10M, CON 50M, CON CM separately as above-mentioned method.

2.4 Synthesis of Gold Nanoparticle (AuNPs):

For the synthesis of AuNPs, 0.01gm of Auri-cloride (HAuCl4) was added to 100 ml of distilled water, then 20 ml of CON Q was added to the solution and vigorously stirred. A change in the colour of solution was observed. Similarly, synthesis of AuNPs was done using other potencies of CON separately.

2.5 Characterization of AgNPs & AuNPs:

The characterization of NPs is important to understand its physical and chemical properties. Nanoparticles have primary external dimension of less than 100 nanometers (nm), and are often known for its unique properties. So, to know whether the bio reduction of AgNPs and AuNPs has happen, the characterization of synthesized AgNPs and AuNPs was done using UV spectroscopy, FTIR analysis, Scanning electron microscopy (SEM) and Transmission Electron Microscopy (TEM).

2.5.1 UV visible Spectroscopy:

UV-vis spectroscopy is one of the reliable techniques for the characterization of synthesized NPs [10]. Each NPs have unique optical properties which make them strongly interact with specific wavelengths of light [11]. In addition, UV-vis spectroscopy is fast, easy, simple, sensitive, selective for different types of NPs, needs only a short period time for measurement, and finally a calibration is not required for particle characterization of colloidal suspensions [12];[13];[14]. In NPs, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band, occurring due to the collective oscillation of electrons of NPs in resonance with the light wave [15];[16];[17];[18];[19];[20]. The absorption of NPs depends on the particle size, dielectric medium, and chemical surroundings [17];[18];[19];[20];[21].

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR):

FTIR of all the samples were done to identify the functional groups. This spectroscopy is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. Even by using FTIR it is possible to detect small absorbance changes, where one could easily distinguish the small absorption bands of functionally active residues from the large background absorption of the entire sample [22];[23];[24];[25];26].

2.5.3 Scanning Electron Microscopy (SEM):

The invention of various high-resolution microscopy techniques in order to learn more about nanomaterials using a beam of highly energetic electrons to probe objects on a very fine scale has made significant advancement in the field of nanotechnology [27]. Among various electron microscopy techniques, SEM analysis resolves different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales [30];[31];[32];[33];[34]. 2.5.4 Transmission Electron Microscopy (TEM):

To study the quantitative measures of nanoparticle size, shape and distribution TEM is the most valuable, frequently used technique now a days [30]. The magnification of TEM is mainly determined by the ratio of the distance between the objective lens and the specimen and the distance between objective lens and its image plane [32]. With comparison to SEM TEM has two advantages 1. It can provide better spatial resolution, 2. It has the capability for additional analytical measurements [33]. Therefore, proper sample preparation is extremely important in order to obtain the highest-quality images possible.

2.6 Antioxidant Potential

Antioxidant property was determined by using DPPH assay. The 1ml test samples were mixed with 3ml of methanolic solution of DPPH with a concentration of 1µg/ml separately. Then the samples were kept in dark room for 30minutes. After 30 minutes each samples absorbance was recorded at 520 nm of colorimeter. As a comparison solution ascorbic acid is used. The scavenging activity % (AA%) measured by using the equation:

$$AA\% = Abs of control - Abs of sample x 100 ----Eq (1)$$

Abs of control

2.7 Antimicrobial Assay Well Diffusion Method

Nutrient Agar media was used in this study. Plated petri dishes using nutrient agar was allowed to solidify for 30 minutes. By using sterile swab, the bacteria were spread on the surface of solidified nutrient agar. Then wells were made by using Cork borer (7mm) after that 20µl of CON Q to CM potencies, synthesized AgNPs and AuNPs samples prepared from different potencies of CON were dispensed into the wells using a micropipette. Ethanol, AgNO3 and HAuCl4 Solutions were used as control group. The prepared samples were allowed to diffuse for 1 hr. at room temperature. After 1hr. the plates were incubated at 37°C for 24 hrs. further diameter of inhibition was measured using scale and then the readings were tabulated.

2.8 Cell Line Study

Cell viability test was done on KB-3-1 cell line using MTT assay protocol.

The reduction of tetrazolium salts is now extensively accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability [40];[41];[42];[43]. **III. RESULTS**

3.1 Phytochemical Analysis

The phytochemical study showed presence of bioactive components such as flavonoids, alkaloids, saponins, tannins, carbohydrates, steroids & Anthroquinons glycosides in CON Q, CON 6C and CON12C. In other potencies of CON such as CON 30C, CON 12C, CON 200, CON 1M, CON 10M, CON 50M, CON CM potencies the presence of phytochemicals were absent. These may be due to these potencies may not be sensitive to the methodology used.

3.2 Visual observation of Synthesized AgNPs

The biosynthesis of AgNPs was visually observed by colour changes occurred after adding 20ml of CON Q and other potencies of CON separately to 80 ml of AgNO3 solution. The Colour of AgNO3 Solution changed within 10 minutes in which CONQ was added. Samples prepared from CON 6, 12, 30,200,1M, 10M, 50M, CM took almost 24 hours in normal room temperature to show light brown colour changes



3.3 Visual observation of Synthesized AuNPs

The biosynthesis of AuNPs was also visually observed by the colour change which occurred after 12 hours of adding CON Q and other potencies in HAuCL4 solution. After 12 hours it changed Dark purple colour in CON Q and light purple Colour in other Potencies. These Colour changes shows the reduction of biomolecules.



3.4 Characterization of AgNPs and AuNPs

3.4.1 UV Visible Spectroscopy

UV visible spectroscopy study confirmed the presence of AgNPs showing absorption peek max in between 420nm to 460nm. AgNPs prepared using CON Q showed peek at 459nm, CON 6 at 454nm, CON 12 at 450nm, CON 30 at 448nm, CON 200 at 446nm, CON 1M at 442nm, CON 10M at 438nm, CON 50M at 434nm and CON CM at 428nm respectively. UV spectroscopic studies of AuNPs showed maximum peek of absorption of 520nm in AuNPs synthesized using CONQ. 3.4.2 *FTIR*

The CON Q to CM potency and Synthesized AgNPs and AuNPs samples may have many components, So FTIR results will reflect the components at different molecular vibration, hence showing differences in molecular vibration spectrum among samples.



Figure.3: FTIR Results of CON Q,6C, 12C, 30C, 200C, 1M, 10M, 50M, CM AgNPs from CON Q,6C, 12C, 30C,

Figure. 4: FTIR Results of Synthesized



Figure.5: FTIR Results of Synthesized AuNPs from CON Q,6C, 12C, 30C, 200C, 1M, 10M, 50M, CM

Table.1 Interpretation of FTIR Result				
Bands cm-1	Component			
3320-3380	-OH, -NH			
2850-2950	C-H stretching (alkanes and alkenes)			
1600	C= C Stretching			
1250-1350	H-O-C, H-C-C			
600-780 C-H bend (alkanes and aromatic ring)				

3.4.3 SEM Analysis of AgNPs

AgNPs synthesized from CON Q to CM showed less than 100nm in size and spherical in shape





3.4.4 TEM Analysis of AuNPs

The TEM analysis of synthesized AuNPs using CON Q to CM exposed the shape and size of nanoparticles, which evidently observed with a size in between of 20 to 100 nm

100 nm	208 nit	<u>100 nm</u>
Figure. 15: AuNPs from CON O	Figure.16: AuNPs from CON6	Figure.17: AuNPs from CON 12
100 nm	50 nm	<u>50 nm</u>
Figure. 18: AuNPs from CON 30	Figure.19: AuNPs from CON 200	Figure. 20: AuNPs from CON 1M
20 mm	<u>20 mm</u>	<u>10 nm</u>
Figure.21: AuNPs from CON	Figure.22: AuNPs from	Figure.23: AuNPs from
10101	CON JUNI	CONCIM

3.5 Antioxidant properties

The antioxidant activity of CON Q and its potencies and Synthesized AgNPs and AuNPs using all the potencies of CON was tested using DPPH assay.

Table.2: Result of Anti-oxidant activity of samples using DPPH assay							
SL.No	Homoeopathic	Reading	%	AgNPs	%	AuNPs	%
	Q& Potencies			Reading		Reading	
1.	Condurango Q	0.04 ± 2	63%	0.04 ± 2	63%	0.07 ± 2	36%
2.	Condurango 6	0.02 ± 2	81%	0.01±2	81%	0.05 ± 2	54%
3.	Condurango 12	0.02 ± 2	81%	0.04±2	63%	0.04 ± 2	63%
4.	Condurango 30	0.02 ± 2	81%	0.06 ± 2	45%	0.05 ± 2	54%
5.	Condurango 200	0.01±2	90%	0.03±2	72%	0.04 ± 2	63%
6.	Condurango 1M	0.01±2	90%	0.06 ± 2	45%	0.06 ± 2	45%
7.	Condurango 10M	0.01±2	90%	0.06 ± 2	45%	0.04 ± 2	63%
8.	Condurango 50M	0.01±2	90%	0.03±2	72%	0.04 ± 2	63%
9.	Condurango CM	0.01±2	90%	0.02±2	81%	0.03±2	72%

3.6 Antimicrobial Activity

Antimicrobial activity study, biosynthesized AgNPs showed maximum diameter of inhibition in comparison with Homoeopathic medicine CON and its Potencies. Diameter of Inhibition was shown less by synthesized AuNPs in comparison with Homoeopathic Medicine CONQ to CM potencies and Synthesized AgNPs.

Tal	Table.3: Diameter of zone of inhibition of CON Q to CM potencies synthesized AgNPs and AuNPs												
SL		Medicine			AgNPs			AuNPs					
NO	Potencies	E.coli ATCC 25922	Staph.aureus ATCC 29213	Lactobacillus MTCC 10307	Kleibsiella.Sp	E.coli ATCC 25922	Staph.aureus ATCC 29213	Lactobacillus MTCC 10307	Kleibsiella.Sp	E.coli ATCC 25922	Staph.aureus ATCC 29213	Lactobacillus MTCC 10307	Kleibsiella.Sp
1	Q	07	08	07	6	13	11	11	10	05	04	04	05
2	6	10	09	06	6	13	10	11	11	04	03	06	05
3	12	06	08	06	7	09	10	10	11	04	04	06	04
4	30	10	08	06	7	10	13	08	11	06	05	05	04
5	200	08	09	06	8	12	10	10	12	06	05	05	04
6	1M	06	11	05	7	13	11	10	12	05	06	05	04
7	10M	06	11	05	9	11	11	08	13	05	04	03	04
8	50M	10	12	06	10	14	12	13	13	04	03	04	03
9	СМ	10	12	06	10	15	12	09	10	05	06	06	06

3.7 Cell Line Study

In all the samples (CON Q to CM potency & biosynthesized AgNPs, and AuNPs) screened for its cytotoxicity against KB-3-1 cell lines at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay.

Table. 4: IC50 (in µg/ml) values of KB-3-1 cell line study in 24hr of CON Q to CM potencies and Synthesised AgNPs, AuNPs							
SL.NO	POTENCIES	KB-3-1 cell line IC ₅₀ (in µg/ml) 24hr					
		Condurango	AgNPs	AuNPs			
1	Q	20.47 µg/ml	12.23 µg/ml	57.01µg/ml			
2	6	4.54 μg/ml	5.14 µg/ml	32.15µg/ml			
3	12	5.75 μg/ml	17.54 µg/ml	26.55µg/ml			
4	30	30.76 µg/ml	21.06 µg/ml	30.47µg/ml			
5	200	13.95 µg/ml	10.40 µg/ml	29.43µg/ml			
6	1M	27.66 µg/ml	20.45 µg/ml	32.14µg/ml			
7	10M	9.21 μg/ml	21.43 µg/ml	28.02µg/ml			
8	50M	4.68 µg/ml	11.50 µg/ml	11.65µg/ml			
9	СМ	7.33 µg/ml	8.55 µg/ml	8.11ug/ml			

Table. 5: IC50 (in µg/ml) values of KB-3-1 cell line study in 24hr of Control group					
SL.No	CONTROL	GROUP			
1	Ethanol	16.32 μg/ml			
2	AgNO3	19.60 µg/ml			
3	HauCL4	15.74µg/ml			

Figure 15 CON Q	Figure 14 CON 4	Figure 17 CONU2	Firme 19, CON20
Figure.15 CON Q	Figure.16 CON 6	Figure.17 CON12	Figure.18. CON30
Figure.19 CON 200	Figure.20 CON 1M	Figure.21 CON 10M	Figure.22 CON 50M
Figure.23 CON CM	Figure.24 AgNPs CONQ	Figure.25 AgNPs CON 6	Figure.26: AgNPs CON12
Figure.27: AgNPs CON	Figure. 28: AgNPs CON 200	Figure. 29: AgNPs CON	Figure. 30: AgNPs CON
Figure. 31 AgNPs CON 50M	Figure. 32 AgNPs CON CM	Figure. 33 AuNPs CONQ	Figure. 34 AuNPs CON 6
Figure. 35 AuNPs CON12	Figure. 36 AuNPs CON 30	Figure. 37 AuNPs CON 200	Figure. 38 AuNPs CON 1M
Figure. 39 AuNPs CON 10M	Figure. 40 AuNPs CON 50M	Figure. 41 AuNPs CON CM	Figure.43 ETHANOL



IV. DISCUSSION

Preparation of Homeopathic medicines is carried out using systematic process called as potentization. Potentization involves serial dilution and succussion or trituration at every stage [44]. Phytochemical Analysis CON Q, 6C,12 C showed the presence of phytochemicals such as saponins, flavonoids, carbohydrates, alkaloids, steroids and anthraquinone glycosides. Other potencies such as CON 30, 200, 1M, 10M, 50M & CM potencies didn't show any positive results in phytochemical analysis. This may be due to these potencies may not be chemically sensitive or reactive. So, the methodology used for phytochemical analysis is not sensitive towards higher dilutions of Homoeopathic medicines. For the higher dilutions of Homoeopathic medicines to know the presence of phytochemicals advanced spectroscopic studies are advisable.

We could find from FTIR analysis presence of functional groups such as -OH, -NH, C-H stretching of alkanes and alkenes, C= C, H-O-C, H-C-C, and aromatic rings. And these functional groups shown in FTIR result might be acting as reducing and caping agents, to stabilize the synthesized AgNPs and AuNPs from CON Q, CON 6C, CON 12C, CON 30C, CON 200C, CON 1M, CON 10M, CON 50M and CON CM potencies.

In this study, we used CON Q to CM potencies for the synthesis of AgNPs and AuNPs. The visual colour changes after the 24 hrs. shows the bio reduction of AgNPs and AuNPs. From UV spectroscopic studies, SEM and TEM analysis of samples confirmed the bio reduction of AgNPs and AuNPs.

After the confirmation of bio reduction of AgNPs and AuNPs, CON Q to all potencies and Synthesized AgNPs and AuNPs were subjected to antioxidant, antimicrobial & Cell line studies.

In this study the strongest antioxidant activity was shown by Homoeopathic drug Condurango in its higher potencies in comparison with other two groups of AgNPs and AuNPs samples. CON Q showed 63% antioxidant property, CON6, 12, 30 showed 81% and CON 200, 1M, 10M, 50M, CM showed 90% of antioxidant property. In Synthesized AgNPs Samples the highest antioxidant Property was shown by AgNPs synthesized using CON 6 & CON CM potencies (81%). Among AuNPs Samples AuNPs synthesized using CON CM potency showed 72% of antioxidant activity. In this study only DPPH Assay was used to study the antioxidant activity.

Antimicrobial study showed highest zone of inhibition in synthesized AgNPs samples in comparison with Homoeopathic drug CON Q to all other potencies and synthesized AuNPs.

In cell line study KB-3-1 cells were treated with different concentration of each sample for 24 hours. IC50 (in μ g/ml) at 24hours shown 50% growth inhibition which shows the effectiveness of Homoeopathic drug Condurango and its all potencies and synthesized AgNPs and AuNPs by it has good anticancer activity.

The best results were seen in CON 6, 12, 50M, CM potency. In biosynthesized AgNPs, AgNPs samples prepared using CON6, CON CM, CON 200 showed good results. In AuNPs synthesized samples using CON CM potency showed best results among other AuNPs prepared from other CON samples.

V. CONCLUSION

This study concludes that CON Q contains flavonoids, Alkaloids, Saponins, carbohydrates, steroids, and anthraquinone glycosides from phytochemical analysis. And these phytochemicals may be responsible for Condurango having anti-cancerous properties. Also, this study suggests that Homoeopathic drug CON Q and its ultra-dilution has the capacity for bio-reduction hence assisting the biosynthesis of AgNPs and AuNPs. And this Bio-reduction of AgNPs and AuNPs was confirmed through UV spectroscopy, SEM and TEM Analysis. FTIR analysis confirmed the presence of functional groups and its phytochemical interaction in the reduction and stabilization of synthesized AgNPs and AuNPs using Homoeopathic medicine CON Q to CON CM potencies. Further Anti-oxidant & anti-microbial studies showed good results in Homoeopathic medicines and biosynthesized AgNPs and AuNPs. From KB 3-1 Cell line study it was proved Homoeopathic drug Condurango and its different potencies, synthesized AgNPs and AuNPs has significant anti-cancerous property. So, this study can be used as a reference for further studies by using other parameters of cell line studies & animal experimental studies.

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