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Antimicrobial effect of silver nanoparticles mediated cosmetic cream and cotton gauze on *Candida* strains

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Abstract: *A simple one step rapid synthetic route is described for the preparation of silver nanoparticles by reduction of silver nitrate (AgNO3) using cotton gauze in aqueous solution and oleic acid which acts as reducing and capping agent. Oleic acid coated silver nanoparticles used as base in cosmetic cream. The formation of silver nanoparticles is assured by characterization with UV/VIS spectroscopy, the absorbance of the silver nanoparticles is observed at $\lambda_{max.}$ = 412 nm. TEM images show that the nanoparticles are spherical in shape with ~ 5–15 nm dimensions. The antimicrobial activity of as synthesized silver nanoparticles is tested against the different of Candida strains the bacterial growth is inhibited by gradual reduction of the concentration of the silver nanoparticles. The results showed thatthe silver nanoparticles displayed antibacterial activity towards different Candida strains. It can be concluded that the effect of antibacterial activity of silver nanoparticles is higher in the cases (cotton gauze and cosmetic cream).*

Keywords: *silver nanoparticles - cotton gauze and cosmetic cream - Candida strains*

I. Introduction:

Nano-materials are at the leading edge of the rapidly developing field of nanotechnology. Nano-materials have been successfully used in nano-chemistry to enhance the immobilization and activity of catalysts, in medical and pharmaceutical nano-engineering for delivery of therapeutic agents, in chronic disease diagnostics, and in sensors. Nano-materials have been also used in clothing and in the food industry to limit bacterial growth **(1).** Nano-materials with antimicrobial properties are particularly effective because of the high surface to size ratio and enhanced surface reactivity of the nano sized antimicrobial agents, making them able to inactivate more microorganisms when compared to higher scale counterparts**(2)**. Various nanoparticles or nano-composite materials have been investigated for their antimicrobial activity as growth inhibitors, antimicrobial agents **(3)**, and antimicrobial carriers **4-5)**.

Research has been intensive in antibacterial material containing different inorganic substances with various natures. Among them, Silver metal have been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. It is well known that, silver metal was slowly changed to silver ions and bound to the outer bacterial cell preventing or minimizing infection with pathogenic bacteria, without cause normal human cells damage **(6-7)**.

Among the various synthetic methods used for the preparation of silver nanoparticles, chemical reducing method by using a reducing agent such as sodium borohydrate, citrate orascorbate in a silver salt solution is most common **(8,9)**. Synthetic reducing agents are normally associated with environmental toxicity or biological hazards. Recently, the incorporation of silver nanoparticles on cotton fibers has received great attention due to their high resistance to microbes. **Duran et al., (10-13)** incorporated silver nanoparticles on cotton fabrics having good antibacterial property by fungal process. **Yu et al., (14)** also reported the incorporation of silver nanoparticles onto ultrafine fibers by electro spinning.

The present study involves the preparation of silver nanoparticles mediated cosmetic cream and cotton gauze. The advantages of this process are no need to have extra reducing agents as well as the process can be conducted in aqueous solution or organic natural medium and finally the developed silver nanoparticles by this process have excellent properties and including long term dispersion stability. The antibacterial property of silver nanoparticles mediated cosmetic cream and cotton gauze were tested against different strains of Candida albicans bacteria.

II. Materials and methods

100% of cotton gauze and cosmetic cream from (pharmacy) were used as substrate for experimental work in this study. Oleic acid purism (fluka), Silver nitrate (AgNO3) purist (fluka) obtained from sigma and double distilled water.

Instrumentation:

The absorption spectra of oleic acid coated silver nanoparticles were obtained using an ultraviolet-visible (UV/VIS) spectrophotometer [Lambda 35, Perkin-Elmer, USA] with narrow slit width, 1 cm optical path quartz cuvette. Transmission electron microscopy [TEM, Hitachi HU-11B)] at a voltage of 80 kV was used to study the particles size, and morphology of Ag NPs. The NPs was drop cast onto a carbon coated copper grid, and the diameter was determined from the micrographs.

1-Preparation of oleic acid coated silver nanoparticles.

1 ml of $5x10^{-3}$ mol dm^{-3} of ($AgNO_3$) was added drop wise to 10 ml of oleic acid at 200 C^o. Heating continued for another 5 minutes after that the solution was removed from the heater and stirred for a further 15 minutes.

2-Preparation of silver nanoparticles coated cotton gauze.

Silver nanoparticles coated cotton gauze was prepared according to the following. 5ml of $5x10^{-3}$ mol dm^{-3} ($AgNO_3$) solution was added to 95 ml of double distilled water in 100 ml beaker with stirring. The solution was heated until it begins to boil. Then 10 g of 100% cotton gauze was immersed as soon as the boiling started. Continue stirring with boiling until the color of cotton gauze changes to faint yellow. The heating was stopped with continue stirring to another 15 minutes.

Microbial activity

The antibacterial activities of silver nanoparticles coated cotton gauze and cosmetic cream loaded with oleic acid coated silver nanoparticles were tested by an inhibition zone method. In this method, different Candida strains were taken as the model bacteria. For this study, the silver nanoparticles coated cotton gauze were cut into small pieces (1mm thickness and 1mm cm length), put together to form a circular zone, and the antimicrobial activity was tested using modified agar diffusion assay (disc test). The plates were examined for possible clear zone formation after incubation at 37 $^{\circ}$C for one day. The presence of clear zone around fibers on the plates was recorded as an inhibition against the Candida strains, the cosmetic, cream loaded with oleic acid coated silver nanoparticles were dropped as circle zone of different concentrations in the plate.

III. Results and Discussion

Spectroscopic characterization

The UV–VIS spectral analysis is carried out with the objective to establish the presence of silver nanoparticles in olic acid. Figure (2) showed that, the spherical silver nanoparticles have only one Plasmon absorption band $\lambda_{max.}$ = 412 nm with a detected broad band observed at longer wavelength of the visible region extended to 900 nm. The broadening of the spectral line may be due some aggregation of the nanoparticles. To get the confirmation of the formation of silver nanoparticles coated by olic acid, high resolution transmission electron microscopic analysis is performed. Figure (3 & 4), represents the TEM micrograph of oleic acid coated Ag nanoparticles. From the figure (4), it is clear that the particles are spherical, and not well homogenous with some agglomeration. It is clear from Figure (3) that the silver nanoparticles are of 5–15 nm and possess an average size of 10 nm. In addition, the selected area electron diffraction (SAED) patterns of olic acid coated Ag nanoparticles shows the crystalline character and ordered orientations of the lattice fringe of the silver nanoparticles.

Antimicrobial activity

Qualitative antimicrobial efficiency of silver nanoparticle mediated cosmetic cream and coated cotton gauze was performed on different Candida strains subjected in the present study. The strains were C. albicans, C. glabrata, C. tropicalis C. kyfer. In the present study the antimicrobial activity of silver nanoparticles with different concentrations were carried out. Zone of inhibition around silver nanoparticles for individual bacterial culture is shown in Figures (5, 6, 8, & 9) the silver nanoparticles displayed antibacterial activity towards different Candida strains. Bacterial growth inhibition around the well is due to the release of diffusible inhibitory compounds from silver nanoparticles. Figure (7) and tables (2,3,4,5,) showed the antimicrobial activity of silver nanoparticles produced from 0.1mg ml^{-1} and 0.4mg ml^{-1} against different Candida strains. These results showed inhibition activity different Candida strains by concentration and passing of the hours. Based on Figure (10) it can be concluded that the effect of antibacterial activity is higher in the cases. These finding compatible with previous studies, **Hong** and **Rhim**[3] showed various nanoparticles or nano-composite materials have been investigated for their antimicrobial activity as growth inhibitors,

antimicrobial agents, and **Rhim, et al., [5]** who study silver nanoparticles composite films with antimicrobial activity and **Yu, et al., [14]** showed inhibitor effect of antibacterial PAN-based hallow fibre loaded with silver nitrate and antimicrobial carriers **[4]. Law, et al., [15]** reported that the silver nanoparticles which are in inert form and also exhibit antimicrobial function by inducing the production of reactive oxygen species such as hydrogen peroxide.

Table (1& 6) showed **i**nhibitory effect of Candida strains as affected by different concentrations of liquid. It clarified there is a positive relationship between enhance in diameter of candida and bacterial growth inhibition with increasing concentration of silver nanoparticles. These results harmonious with **Duran et al., [13]** who reported that silver nanoparticles on cotton fabrics having good antibacterial property by fungal process. **Sondi, and Salopek-Sondi [16]** said that silver is generally used in the nitrate form to induce antimicrobial effect, but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbe to be exposed to. Though silver nanoparticles find use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition.

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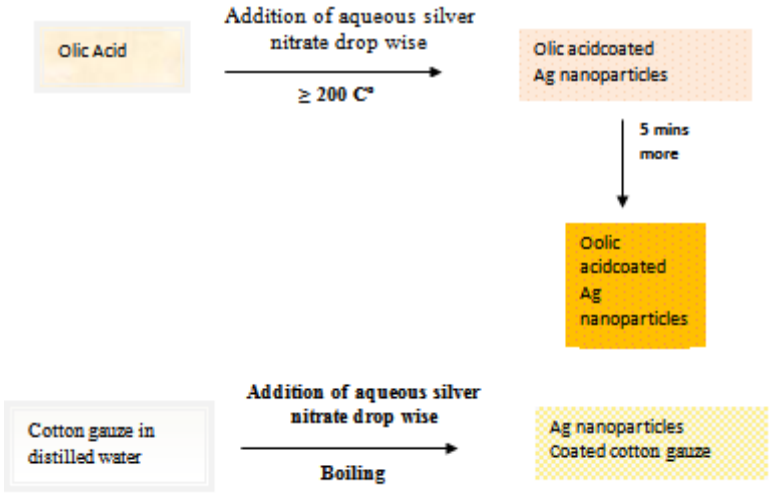


Figure (1): Ag nanoparticles coated with Olic acid

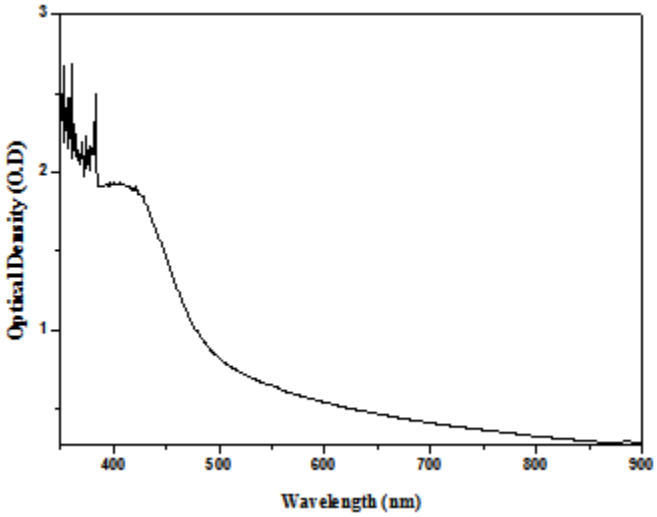
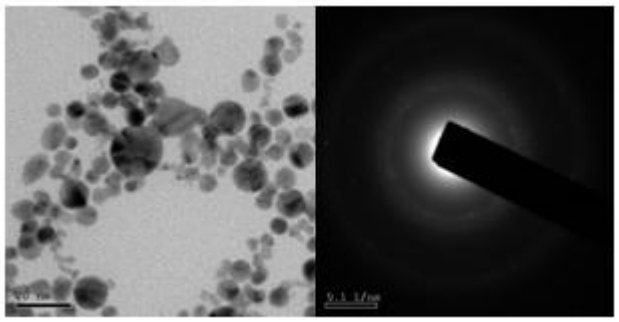
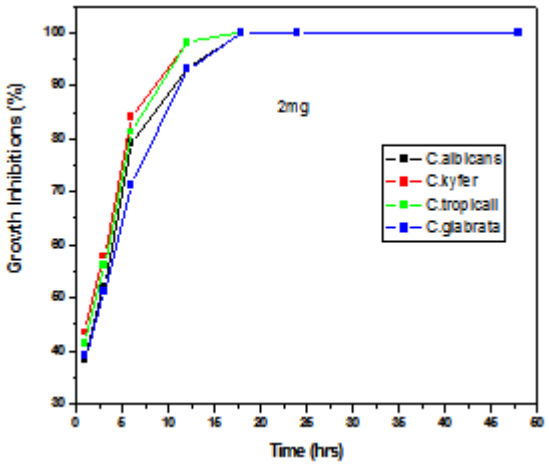


Figure (2) the spherical silver nanoparticles have only one Plasmon absorption band $\lambda_{max.}$ = 412 nm



Figures (3&4) Transmission electron microscopy



Figur (5) silver nanoparticles displayed antibacterial activity towards different Candida strains

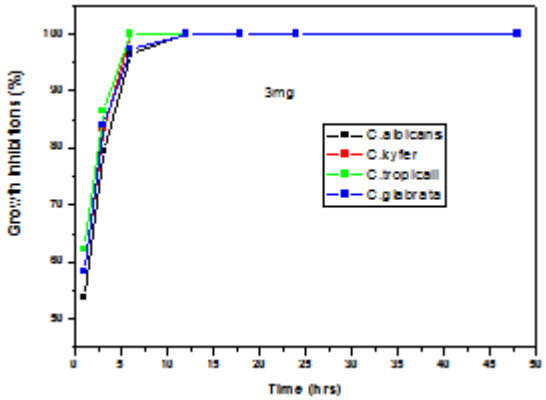


Figure (6) silver nanoparticles displayed antibacterial activity towards different Candida strains

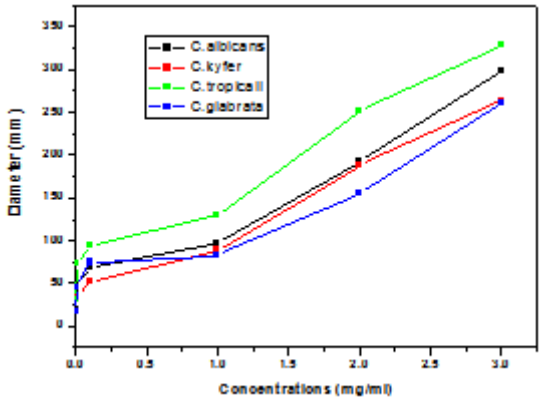


Figure (7) the effect of antibacterial activity is higher in the cases.

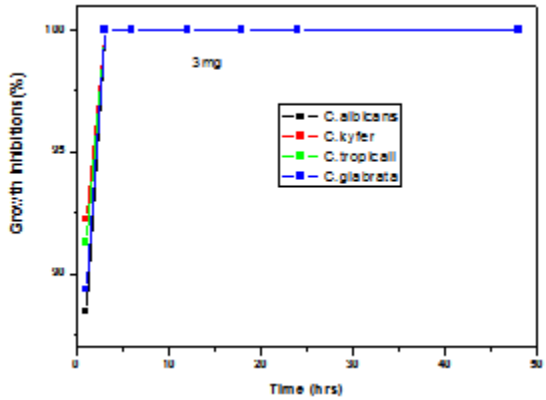


Figure (8) silver nanoparticles displayed antibacterial activity towards different Candida strains

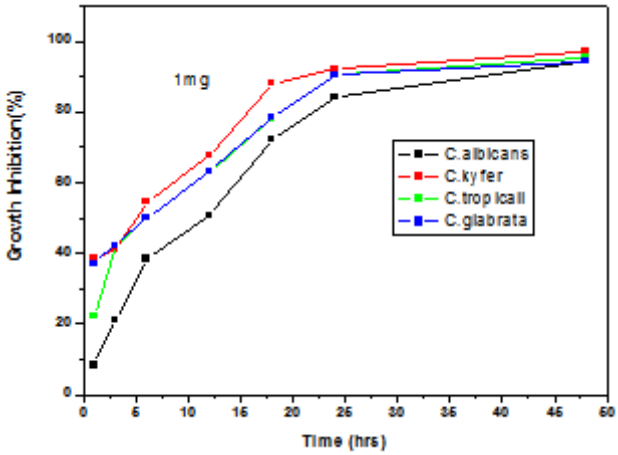


Figure (9) silver nanoparticles displayed antibacterial activity towards different Candida strains

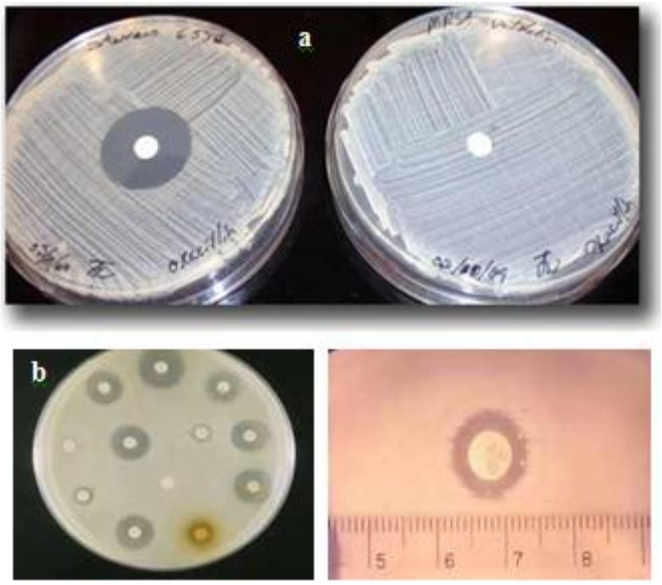


Figure (10) Zone of Inhibitory effect of Candida strain**: (a)** silver nanoparticles coated cotton gauze, and **(b)** silver nanoparticles mediated cosmetic cream.

Table 1 - Inhibitory effect of Candida strains as affected by different concentrations of liquid

Concentration (mg/ml)	Diameter of Candida and bacterial growth inhibition zone (mm)			
	C. albicans	**C. glabrata**	**C. tropicalis**	**C. kyfer**
0.001	19.15 ± 0.06	18. 11 ± 0.02	30.35 ± 0.13	17.42 ± 8.22
0.01	49.55 ± 2.12	35.04 ± 2.12	72.20 ± 4.33	46.33 ± 6.12
0. 1	68.28 ± 8.45	52.35 ± 4.30	93.66 ± 2.57	74.60 ± 2.50
1.0	96.48 ± 15.25	88.41 ± 5.27	130.25 ± 4.37	82.66 ± 5.45
2.0	193.24 ± 17.33	188.60 ± 5.39	252.45 ± 10.10	155.22 ± 6.15
3.0	298.27 ± 24.16	264.17 ± 34.39	328.25 ± 21.22	261.45 ± 25.10

Table 2- Growth inhibition (%) of tested organisms as affected by 1 mg/ml of liquid sample

Time (hours)	C. albicans	C. kyfer	C. tropicalis	C. glabrata
1	08.33 ± 1.02	38.55 ± 1.04	22.22 ± 1.03	94.35 ± 12.05
3	21.11 ± 1.02	41.15 ± 2.04	42.10 ± 3.03	90.50 ± 10.05
6	38.40 ± 2.02	54.50 ± 3.04	50.13 ± 3.03	78.55 ± 10.02
12	50.56 ± 2.02	67.55 ± 5.06	63.11 ± 6.03	63.20 ± 8.03
18	72.12 ± 9.05	88.10 ± 7.021	78.23 ± 7.02	50.10 ± 6.03
24	84.12 ± 13.01	92.12 ±10.02	90.45 ± 11.05	42.15 ± 4.03
48	94.44 ± 15.01	97.33 ± 11.02	95.10 ± 9.05	37.10 ± 5.03

Table 3- Growth inhibition (%) of tested organisms as affected by 2 mg/ml of liquid sample

Time (hours)	C. albicans	C. kyfer	C. tropicalis	C. glabrata
1	38.26 ± 2.11	43.58 ± 3.25	41.45 ± 5.22	39.20 ± 8.11
3	52.24 ± 3.41	57.90 ± 6.66	56.15 ± 8.22	51.22 ± 6.11
6	79.27 ± 4.15	84.10 ± 5.12	81.27 ± 6.12	71.20 ± 6.33
12	93.22 ± 6.33	98.12 ± 9.33	98.10 ± 4.21	93.11 ± 7.25
18	100	100	100	100
24	100	100	100	100
48	100	100	100	100

Table 4- Growth inhibition (%) of tested organisms as affected by 3 mg/ml of liquid sample

Time (hours)	C. albicans	C. kyfer	C. tropicalis	C. glabrata
1	53.66 ± 7.15	58.23 ± 8.11	62.37 ± 4.35	58.36 ± 6.24
3	79.55 ± 9.20	83.33 ± 7.25	86.46 ± 12.35	84.15 ± 4.33
6	96.46 ± 11.10	100	100	97.36 ± 9.46
12	100	100	100	100
18	100	100	100	100
24	100	100	100	100
48	100	100	100	100

Table 5- Growth inhibition (%) of tested organisms as affected by 4 mg/ml of liquid sample

Time (hours)	C. albicans	C. kyfer	C. tropicalis	C. glabrata
1	88.46 ± 12.13	92.26 ± 9.20	91.30 ± 10.25	89.35 ± 9.34
3	100	100	100	100
6	100	100	100	100
12	100	100	100	100
18	100	100	100	100
24	100	100	100	100
48	100	100	100	100

Table 6- Cell proliferation rate (cell density) of cell culture with different concentrations of nanoparticles

Concentration (ng/ml)	1st hour	2nd hour	3rd hour	4th hour
2	$1.8x10^5$	2.3x105	1.8x105	2.3x105
4	2.2x105	1.9x105	1.8x105	1.2x105
6	2.4x105	1.7x105	2.4x105	2.2x105
8	1.9x105	2.5x105	2.3x105	2.5x105
10	1.9x105	2.4x105	2.4x105	1.8x105
12	2.2x105	2.5x105	2.2x105	2.1x105
14	2.4x105	2.5x105	2.4x105	2.1x105