ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Citrullus Colocynthis Nano Silver –Hybrid as Antimicrobial Agent

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Manuscript Info

Manuscript History:

Received: 22 March 2015
Final Accepted: 19 April 2015
Published Online: May 2015

Key words:

Citrullus Colocynthis, silver nanoparticles, antimicrobial activity, wool and viscose fabric, (UV-TEM, SEM, EDXA)

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Abstract

Silver nanoparticles colloidal solutions (AgNPs) was prepared by using *Citrullus Colocynthis* (*C.Colocynthis*) as reducing and stabilizing agent. Characterizations of the prepared solutions were carried out by ultraviolet-visible (UV- Vis) spectra for estimation peaks of AgNPs. These peaks appeared at 420. Transmutation Electron Microscopes (TEM) for determination of size, shape and size distribution of AgNPs were investigated. It is observed that the average of size nanoparticles is use in between 2.04 - 12.64. Wool and viscose fabrics was treated with the prepared AgNPs colloidal solution by padding method to accept them antimicrobial properties. The changes in yellowness, tensile strength, elongation at break and moisture regain have been studied .The treated fabrics were evaluated via monitoring morphological changes of the fibers using scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX) . The phenolic compounds in fruit were also qualitative and quantitative determined using HPLC.

INTRODUCTION

The using of chemical pesticides to get rid of the bacteria to the emergence of many problems such as pollution of the environment and the appearance of generations from insects is resist to pesticides. For these reasons recently, the attention of researchers headed to use of natural plant material. Many laboratory experiments which dealt with the evaluation of the effectiveness of plant extracts in the fight against various microbes. *Citrullus Colocynthis* (L.) is an important medical plant belonging to the family *Cucurbitaceae*. It is an annual herb widely distributed in Mediterranean strip **(1).** *Citrullus Colocynthis (L.) (Cucurbitaceae*), ordinarily known as bitter apple, locally called *Hinzel*. *C.Colocynthis* has gained increasing attention as a natural insecticide and its activity has been evaluated against many economically important insect species **(2, 3)**. Also, it is used as a purgative and vermifuge, and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, and tumors and as an insect repellent **(4*).*** *Citrullus Colocynthis* fruits were extracted by different solvents (n- hexane, methylene chloride, chloroform and ethanol). Mass analysis of the isolated and purified compound showed the molecular ion peak at m/z 719. The element analysis (C, H and N) suggesting the molecular formula to be $C_{38} H_{55} O_{13}$ **(5)**. Silver nano is a trademark name of an antibacterial technology. Antimicrobial effect of silver nanoparticles on textiles has already been shown by various researchers **(6-8).** Silver nano colloids have been synthesized by chemical reduction of silver salt solution. Also Ag nano treatments improve the resistance to microbial attack **(9, 11**). The use of non-toxic chemicals, environmentally eco-friendly and renewable materials are some of the key issues that should be considered in such strategy **(12**).Synthesis of silver nanoparticles is an increasing commercial demand due to the wide applicability in various areas for example catalysis, chemistry, cosmetics, and medicine. Recently, synthesis of silver nanoparticles has been investigated by using leaf extracts of *Citrullus Colocynthis* (L.) **(13).** The present study, silver nanoparticles colloidal solutions (AgNPs) were prepared from *Citrullus Colocynthis* (either a whole fruit or separate seeds or fruit without seeds) as reducing and stabilizing. Wool and viscose fabrics was treated with the prepared AgNPs colloidal solutions to accept them antimicrobial against Gram-positive bacteria, Gram-negative bacteria, Yeast and Fungi

Experimental

Materials &Methods

Materials

Citrullus Colocynthis (*C.Colocynthis*) (*Hanzal* fruits) were collected from South Sinai in Egypt. The ripe fruits are marbled green with white and yellowish patches but color unripe fruits are green. Silver nitrate was purchased from Sigma - Aldrich, Germany (ACS reagent, ≥99.0%Ag NO_3) . All other chemicals and reagents used were laboratory grade. Wool and viscose were supplied by Misr – El Mahalla Co., Egypt. Nonionic detergent (nonyl phenol ethoxylane, was supplied from Starch & Detergent Company. Alexandria, Egypt).

Methods

The ethanolic extract obtained from dried powder fruits (C.Colocynthis)

Fruits were washed with water and dried at room temperature very well. Air dried powdered for a whole fruit, separate seeds and fruit without seeds were grinded separately and extracted by maceration in ethanol – water (4:1, v/v) at room temperature for three successive times. The combined extracts were then filtered and concentrated under reduced pressure at 45ºC to obtain the ethanolic crude extract.

Synthesis of Silver Nanoparticles (AgNPs) using (C.Colocynthis)

Put dried powdered fruit of *Citrullus Colocynthis* (either a whole fruit or separate seeds or fruit without seeds) which prepared in the preceding steep, in conical flask then add100 ml sodium hydroxide (0.01N), all this system keep under magnetic stirring at temperature 80^0C then add silver nitrate (25,50and100ppm) drop wise till the brown color appear, the stirring continues for 30 minutes.

Sample preparation for phenolic compounds identification by HPLC

Fruits *(C.Colocynthis***)** were extracted according to Kim *et al*. (2006). Briefly, 10 mg was submitted to an alkaline hydrolysis by shaking with 200 mL of 2 M NaOH, then PH was adjusted to 2 with 6 M HCL, then filtered. The supernatant was collected, and then extracted twice with ethyl ether and ethyl acetate. The organic phase was separated and evaporated. The residue was extracted by sonication with methanol 80% at room temperature, the extract was filtered, clarified by centrifugation. Pure standards were used as external standards to identify the compounds (All standards were purchased from Sigma- Aldrich, Germany). Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

Treatment of fabrics with the prepared AgNPs colloidal solution

Wool was washed by using 1g / l nonionic detergent and 1 g/l sodium carbonate at 50 º C for 30 min., L.R 1:40 (O.W.F) . Viscous was soaped with 2g/l nonionic detergent at 60 º C for 45min., L.R. 1:25. Then rinsed thoroughly with cold water and dried at room temperature. Wool and viscose fabrics were treated with the specific concentrations (25,50,100ppm)of the prepared AgNPs colloidal solutions (either a whole fruit or separate seeds or fruit without seeds)and 1% citric acid by padding technique at liquor ratio 1: 10 (o.w.f) for 1 h ., pick up 100%. The fabrics were dried at room temperature and cured at 120 º C for 3min .Then rinsed thoroughly with running water and dried at room temperature.

Measurements and analysis

Ultraviolet-visible (UV-vis) Spectral

UV-vis spectra have been proved to be quite sensitive to the formation of silver colloids because AgNPs exhibit an intense absorption peak due to the surface Plasmon excitation which describes the collective excitation of conductive electrons in a metal. The prepared silver nanoparticles (AgNPs) colloidal solutions were recorded in spectra 50 Analytika Jena Spectrophotometer - Japan from 300 to 550. Distilled water was used as the blank.

Transmission Electron Microscopy (TEM)

Shape and size of the prepared AgNPs colloidal solutions were practically determined using TEM; JEOL-JEM-1200-Japan. Specimens for TEM measurements were prepared by placing a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of the prepared AgNPs colloidal solutions was determined from the diameter of 100 nanoparticles found in several arbitrarily chosen areas in enlarged microphotographs.

Antimicrobial activity

Pathogenic organisms

All the tested organisms were supplied by the Biotechnological Research Center, Al-Azhar University (Cairo, Egypt); the bacteria were maintained on NAM and the yeast on potato dextrose agar (PDA) medium (peeled potato 200 g, dextrose 20 g, agar 18 g in 1000 ml distilled water). Gram-positive bacteria comprised *Bacillus subtilis* (NCTC 1040), *Bacillus megaterium*, *Bacillus cereus and Staphylococcus aureus* (NCTC 7447). *Gram-negative* bacteria comprised *Escherichia coli* (NCTC 10416), *Pseudomonas aeruginosa* (ATCC 10145); *Enterobacter cloacae and Klebsiella pneumonia*. The yeast was *Candida albicans* (IMRU 3669)*; and Saccharomyces cerevisiae*. Fungi were *Aspergillus niger*. Both media were previously inoculated with a 0.1 ml spore suspension of the pathogens.The previously mentioned bacteria were incubated overnight at 37°C for 24 h, whereas yeast was cultured at 28°C for 4 days. The inhibition zones were measured by the treated fabrics with the prepared AgNPs colloidal solutions in mm to select the most active of them **(14).**

Yellowness Index

Measurement of yellowness index was determined using Ultrascan Prospector Photometer (Hunter Lab) Made in USA.

Tensile Strength and Elongation

The tensile properties of wool and viscose fabrics before and after treatment with AgNPs colloidal solutions were investigated using an Instron Tensile Tester (USA) according to ASTM D 76 Standard Specification for Textile Testing Machines

Moisture Regain

The measurement of the moisture regain of wool and viscose fabrics before and after treatment with AgNPs colloidal solutions were performed according to ASTM Standard 2654-76(1981). The moisture regain of the samples were calculated according to:

$$\text{Moisture regain } \% = \frac{W_1 - W_2}{W_2} \text{ x } 100$$

Where

W_1: Weight of sample a

W_2: Constant weight of dried sample.

Scanning Electron microscopy (SEM) & Energy dispersive X-ray analysis (EDAX)

SEM & *EDAX* of the treated fabrics was studied using a Scanning Electron Probe Microanalyses (type JXA-840A) – Japan. The specimens in the form of fabrics were mounted on the specimen stabs and coated with thin film of gold by the sputtering method. The micrographs were taken at two magnifications, namely 1000 and 2500, using 30 kV accelerating voltage

Results and Discussion

Ultraviolet-visible (UV-vis) Spectral

Figure2 shows UV-vis absorption spectroscopy for concentration (100 ppm) of the prepared AgNPs colloidal solutions (either a whole fruit or separate seeds or fruit without seeds).

It is observed that where the electronic absorption spectra of curves (a, b & c) for AgNPs colloidal solutions (either a whole fruit or separate seeds or fruit without seeds) respectively give a sharp absorption intensity of Plasmon peak indicating reduction of Ag^+ to Ag^0 as evidenced by the appearance of Plasmon peak at 420nm.But the intensity give higher one for AgNPs colloidal solutions of whole fruit curve (a) and the lower one for AgNPs colloidal solutions of whole fruit without seeds curve (c) and the AgNPs colloidal solutions formed with seeds only give intensity with moderate value curve (b).

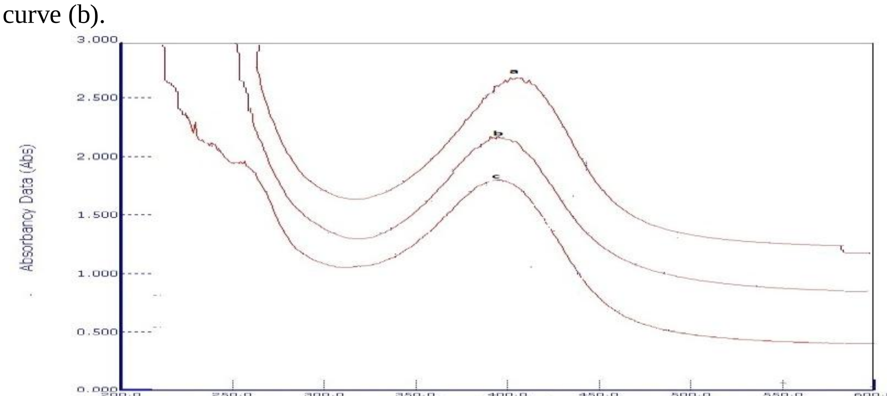


Figure2: UV-vis absorption spectroscopy for concentration (100 ppm) of the prepared AgNPs colloidal solutions Where: a, b, c (either awhole fruit or separate seeds or fruit without seeds respectively

Figure 3and 4 show UV-vis absorption spectroscopy for AgNPs colloidal solution prepared using *(C. Colocynthis*) as reducing and stabilizing at concentration (50 and 25ppm) of silver nitrate ,where the electronic absorption spectra of curves (a, b & c) for a whole fruit, separate seeds and fruit without seeds respectively. It is observed that intensity of Plasmon peak with whole fruit curve (a) at 50and 25ppm concentrations give higher intensity peak with whole fruit than the other component curve (b, c) with two concentrations of silver nitrate, but the intensity of peaks still lower than the concentration of silver nitrate (100ppm) in figure 2, this indicate that the formation of silver nanoparticles are sensitively related to the presences of the seeds and the intensity of peak increase with increasing silver nitrate concentration.

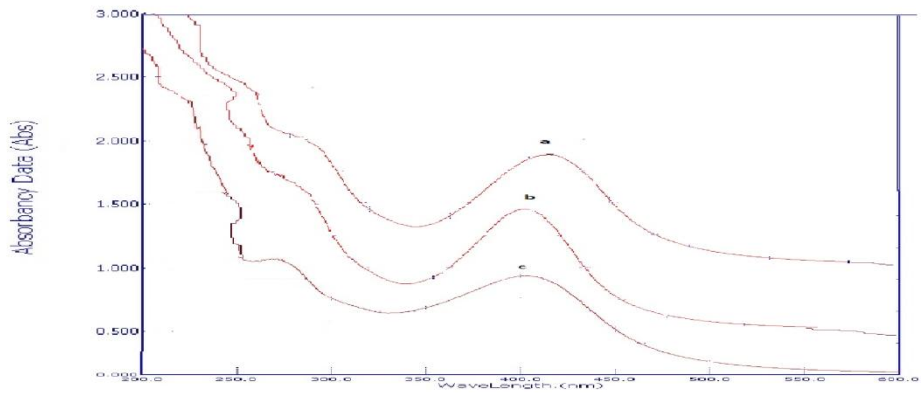


Figure3: UV-vis absorption spectroscopy for concentration (50 ppm) of the prepared AgNPs colloidal solutions Where: a, b, c (either a whole fruit or separate seeds or fruit without seeds respectively

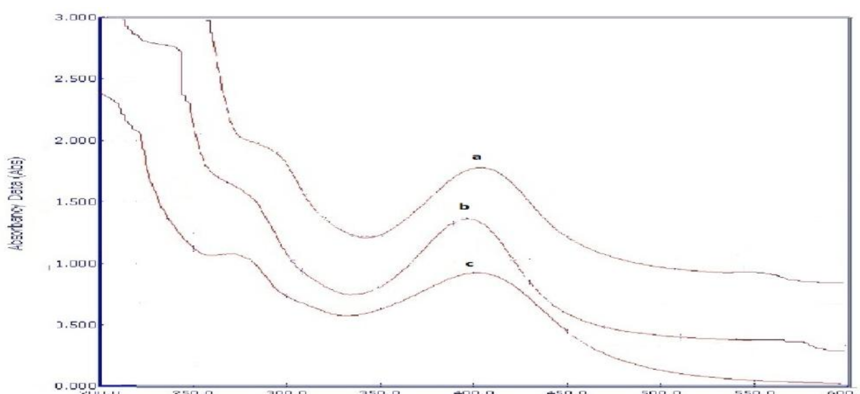
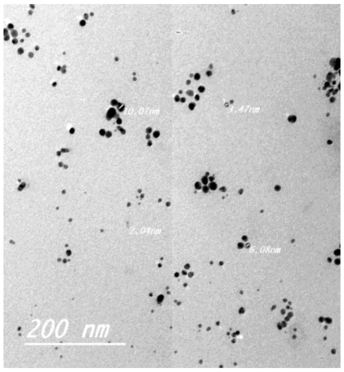


Figure 4: UV-vis absorption spectroscopy for concentration (25 ppm) of the prepared AgNPs colloidal solutions Where: a, b, c (either awhole fruit or separate seeds or fruit without seeds respectively

Transmission Electron Microscopy (TEM)

Figures 5, 6, 7 show the TEM images and their corresponding particle size distributions of
AgNPs colloidal solution prepared using *(C. Colocynthis) at* concentration (100ppm) of silver nitrate (either a whole fruit or separate seeds or fruit without seeds respectively). This indicates that the TEM of AgNPs particles of fruit without seeds is not homogenous and coagulated and their shape is not well-defined as shown in figure 7. Also it is observed that the TEM of AgNPs prepared using a whole fruit and seeds exhibit spherical shape with enough amount diameter ranging from 2-10nm, in addition to being homogenous and not coagulated. These results confirm the presence of whole fruit and seeds in the preparation of AgNPs are important and produce particles with ideal size and morphology.



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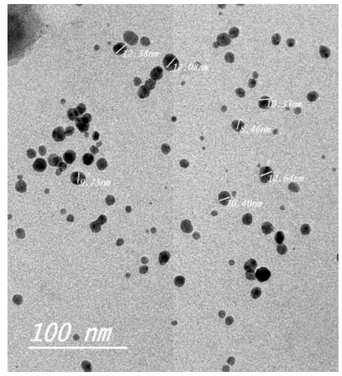

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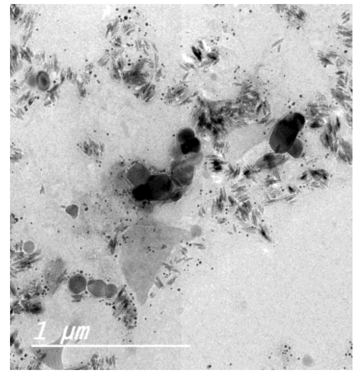

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Figures 5-7: Transmission Electron Microscopy (TEM) of the prepared AgNPs colloidal solutions at concentration (100ppm) (either a whole fruit or separate seeds or fruit without seeds respectively)

Antimicrobial active

Tables 2, 3 show the inhibition zone diameter (mm/1 cm sample) for all the tested organisms on the untreated and treated each of wool & viscose fabrics. The antimicrobial activity was observed with the treated wool & viscose fabrics than the untreated fabrics. The highest antimicrobial activity was imparted upon treatment with AgNPs colloidal solutions were prepared from a whole fruit with 100ppm concentration against *Staphylococcus aureus (*G+), Enterobacter *cloacae (*G-) *and Candida albicans* (Yeast).

Table 2: Antimicrobial activity of the untreated and treated wool fabrics with the prepared AgNPs colloidal solutions (either a whole fruit or separate seeds or fruit without seeds respectively) at concentration 100, 50,25ppm

Bacterial species Samples	**Untreated wool**	**Wool treated with a whole fruit**			**Wool treated with Separate seeds**			**Wool treated with fruit without seeds**		
		100ppm	50ppm	25ppm	100ppm	50ppm	25ppm	100ppm	50ppm	25ppm
	Inhibition Zone Diameter (mm/1 cm sample)									
Gram-positive bacteria:										
-Bacillus subtilis	0	10	9	6	9	6	6	5	4	4
-Bacillus megaterium	0	7	6	4	6	5	5	5	3	4
-Bacillus cereus	0	15	10	6	12	11	8	5	4	4
-Staphylococcus aureus	0	17	15	13	12	11	9	6	6	5
***Gram-negative* bacteria:**										
-Escherichia coli	0	10	8	6	8	7	6	7	3	3
-Pseudomonas aeruginosa	0	10	8	6	8	7	6	6	3	3
-Enterobacter cloacae	0	15	12	12	11	9	8	6	6	5
-Klebsiella pneumonia	0	13	10	7	9	9	8	6	4	4
Yeast:										
-Candida albicans	0	14	11	10	9	8	7	6	6	5
-Saccharomyces cerevisiae	0	0	0	0	0	0	0	0	0	0
Fungi :										
-Aspergillus niger	0	0	0	0	0	0	0	0	0	0

Table 3: Antimicrobial activity of the untreated and treated viscose fabrics with the prepared AgNPs colloidal solutions (either a whole fruit or separate seeds or fruit without seeds respectively) at concentration 100, 50,25ppm

Bacterial species / Samples	Untreated viscose	Viscose treated with a whole fruit			Viscose treated with Separate seeds			Viscose treated with fruit without seeds		
		100ppm	50ppm	25ppm	100ppm	50ppm	25ppm	100ppm	50ppm	25ppm
	Inhibition Zone Diameter (mm/1 cm sample)									
Gram-positive bacteria:										
-Bacillus subtilis	0	8	8	7	9	8	8	5	5	4
-Bacillus megaterium	0	7	6	6	6	5	4	5	3	4
-Bacillus cereus	0	11	9	7	10	9	8	5	4	4
-Staphylococcus aureus	0	14	13	11	12	11	9	6	6	5
***Gram-negative* bacteria:**										
-Escherichia coli	0	10	8	6	8	7	6	7	3	3
-Pseudomonas aeruginosa	0	10	8	6	8	7	6	6	3	3
-Enterobacter cloacae	0	14	12	12	11	9	8	6	6	5
-Klebsiella pneumonia	0	13	10	8	10	9	7	6	4	4
Yeast:										
-Candida albicans	0	12	10	10	9	8	7	7	6	5
-Saccharomyces cerevisiae	0	0	0	0	0	0	0	0	0	0
Fungi :										
-Aspergillus niger	0	0	0	0	0	0	0	0	0	0

Table 4 shows the inhibition zone diameter (mm/1 cm sample) for *Staphylococcus aureus (*G+), Enterobacter *cloacae (*G-) *and Candida albicans* (Yeast) on the untreated and treated each of wool & viscose fabrics with the prepared AgNPs colloidal solutions which prepared from a whole fruit (*C. Colocynthis*),after 1,5,10 washing cycle . It was found that washing led to some decrease in antimicrobial activity of the treated fabrics as compared with the untreated fabrics. This may be due to the fact that metallic ions and metallic compound display a certain degree of sterilizing effect. Nano silver particles have an extremely large relative surface area, thus increasing their contact with bacteria or fungi and vastly improving their bactericidal and fungicidal effectiveness .Nanosilver is reactive with protein organism when contact with bacteria and fungus, it attacks cellular metabolism and inhibits cell growth respiration which lead to death of organisms. In figure 1 and table 5, confirmed this results because the presence of phenolic extracts of fruits (*C. Colocynthis*) that commonly inhibited the growth of selected organism. And the presence of phenolic hydroxyl groups enables phenolic compounds to form coplexes with protein and to a lesser extent with pectin and cellulose. This complexes shows antiviral, antibacterial and antiparasetic effect **(15).**

Table 4: Antimicrobial activity of the untreated and treated wool and viscose fabrics with the prepared AgNPs colloidal solutions which prepared from a whole fruit (*C.Colocynthis*), at 100 ppm concentration after washing for one, five and ten cycle.

Samples	Inhibition Zone Diameter (mm/1 cm sample)								
	Enterobacter cloacae **(G-)**			***Staphylococcus aureus*** **(G+)**			***Candida albicans*** **(Yeast)**		
	1 washing cycle	5 washing cycle	10 washing cycle	1 washing cycle	5 washing cycle	10 washing cycl	1 washing cycl	5 washing cycl	10 washing cycle

						e	e	e	
Untreated wool	0	0	0	0	0	0	0	0	0
Treated wool 100ppm	15	12	13	13	13	10	13	13	8
Treated wool 50ppm	10	8	5	5	5	5	5	6	5
Treated wool 25ppm	4	4	4	4	4	4	4	4	4
Untreated viscose	0	0	0	0	0	0	0	0	0
Treated viscose 100ppm	10	9	10	10	10	8	10	6	6
Treated viscose 50ppm	10	9	8	8	8	8	8	8	6
Treated viscose 25ppm	8	9	7	7	7	8	7	8	6

Determination of the phenolic compounds for (C.Colocynthis) by HPLC is shown in figure 1:

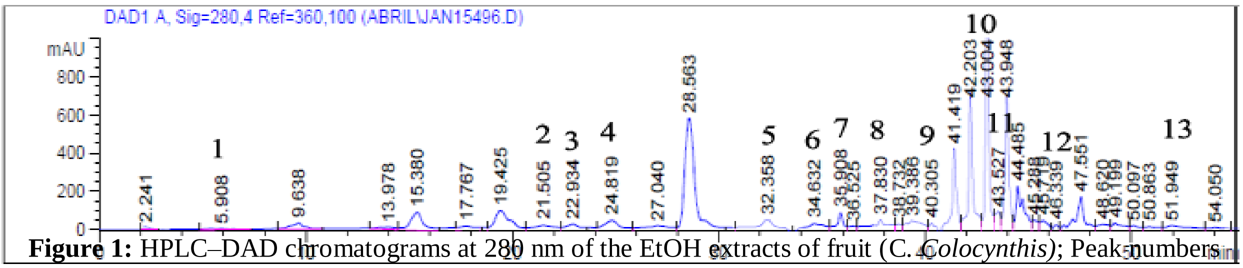


Figure 1: HPLC–DAD chromatograms at 280 nm of the EtOH extracts of fruit (C. *Colocynthis)*; Peak numbers refer to identified phenolics

Table 1: The main constituents in the dry fruits after he HPLC of the fruit (C. *Colocynthis)*

The number of peak	The name of the main constituents	
1	gallic acid	(0.73 μg/g)
2	caffeic acid	(33.82 μg/g)
3	syringic acid	(78.63 μg/g)
4	vanillic acid	(125.9 μg/g)
5	ferulic acid	(75.59 μg/g)
6	sinapic acid	(103.03 μg/g)
7	rutin	(23.67 μg/g)
8	coumarin	(0.31 μg/g)
9	rosmarinic acid	(37.94 μg/g)
10	cinnamic acid	(101.87 μg/g)
11	qurecetin	(4.18 μg/g)
12	Kaempferol	(15.49 μg/g)
13	chrysin	(17.84 μg/g)

Physical and mechanical analysis

The effect of AgNPs colloidal solution prepared from *C. Colocynthis* on the physical and mechanical properties of wool and viscose fabrics are shown in table 5**.** It is observed that moisture regain percentage for treated wool viscose decrease that untreated one. This is may be attributed to reaction between the silver ions with anionic wool group. The yellowness of the treated wool with AgNPs colloidal solution prepared from *C. Colocynthis* was increased as compared to the untreated fabrics. This may be attributed to the natural yellowness color in *hanzal* fruit. On the other hand, there is little enhancement of the Tensile strength of the treated fiber than the untreated one. But it is found that decrease of elongation for treated fabrics compeered with untreated fabrics. This may be attributed to the presence of AgNPs that play an important role in this change.

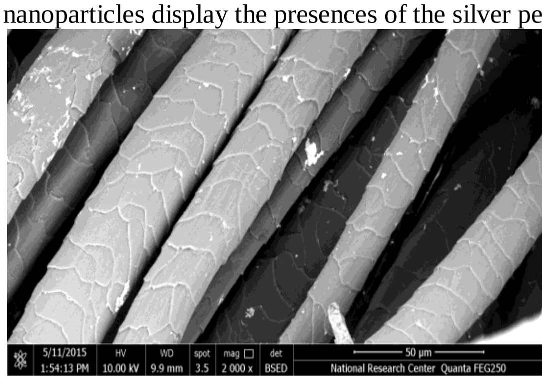
Table 5: Yellowness, Tensile strength, Elongation % and Moisture regain % of untreated and treated wool, viscose fabrics

Samples	Yellowness	Tensile strength	Elongation %

Moisture regain %			(kgf/mm2)	
-Untreated wool	30	1.7	33.5	14.0
- Treated wool	39	2.1	28.0	10.1
- Untreated viscose	0	1.9	21.8	12.0
- Treated viscose	10	2.4	19.0	8.3

Scanning Electron microscopy (SEM) & Energy dispersive X-ray analysis (EDXA) EDAX

The interaction between fabrics and AgNPs are determined by SEM micrographs. Figures 8-11 show scanning electron microscope (SEM) images of wool and viscose before and after treatment with AgNPs at concentration 100ppm of silver nitrate which formed by *C.Colocynthis* (whole fruits) as reducing and stabilizing agent. Figures 8,10 show that the untreated wool and viscose fabrics are characterized by smooth surface without any disturbed structure and no particles present at all, on the other hand ,figures 9,11 show the fabrics treated with the solution containing 100ppm of AgNPs exhibit silver nano particles deposited on the surface of the fabrics .EDXA analysis confirm the presence of silver nanoparticles on the treated fabrics as shown in figures 13,15 where silver nanoparticles display the presences of the silver peak which disappear in the untreated fabrics figure 12,14.



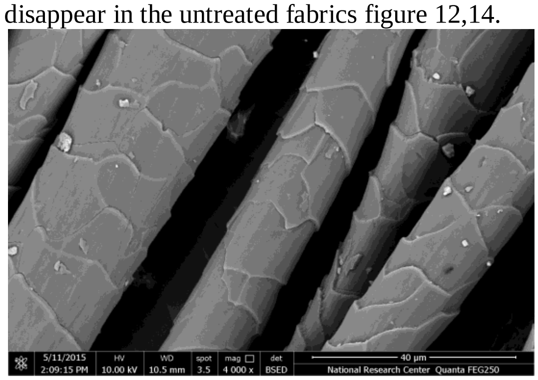
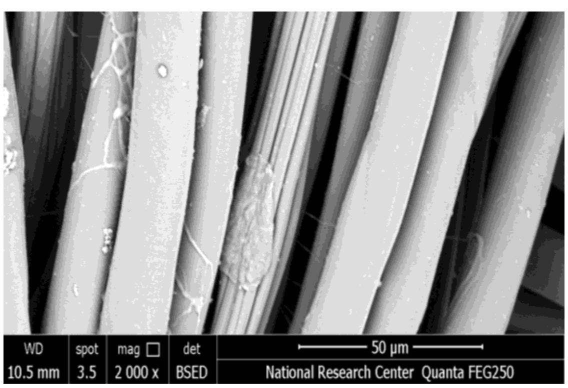


Figure 8: *Scanning electron micrograph of untreated wool* **Figure 9:** *Scanning electron micrograph of wool treated with AgNPs colloidal solution were prepared from a whole fruit (C. Colocynthis) at 100ppm concentration of silver nitrate*



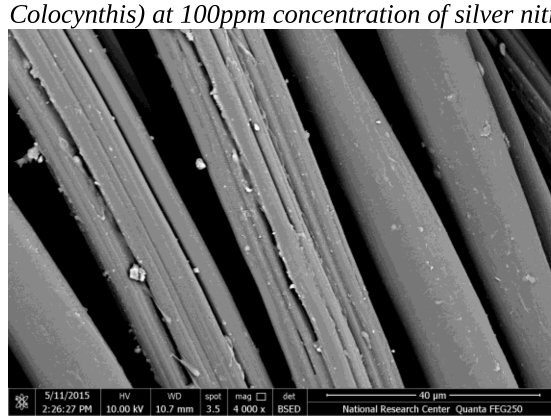


Figure 10: Scanning electron micrograph of untreated viscose **Figure 11:** *Scanning electron micrograph of viscose treated* Ag NPs colloidal solution which prepared from a whole fruit (*C.Colocynthis*) at 100ppm concentration of silver nitrate*with*

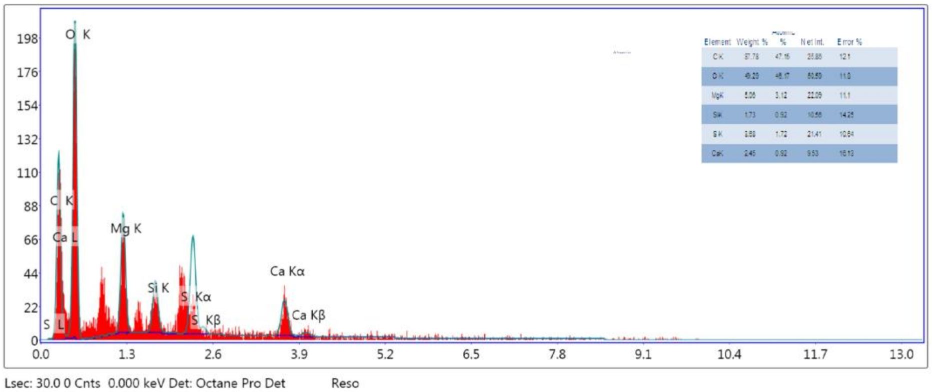


Figure 12: *EDXA analysis of untreated wool*

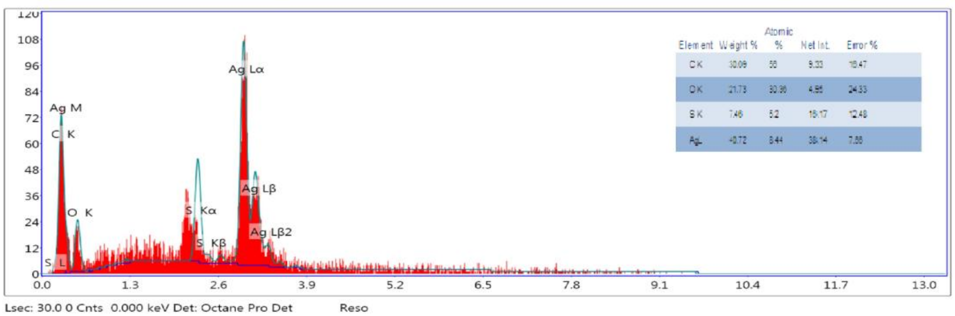


Figure 13: *EDXA analysis of treated wool with AgNPs colloidal solution which prepared from a whole fruit (C.Colocynthis) at 100ppm concentration* of silver nitrate

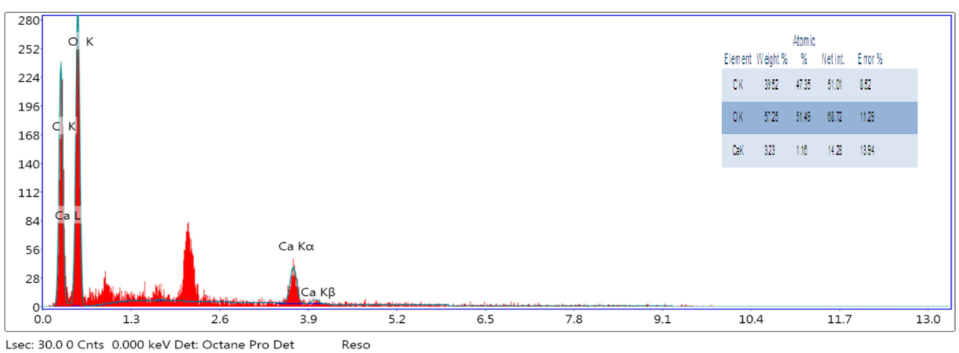


Figure 14: *EDXA analysis of untreated viscose*

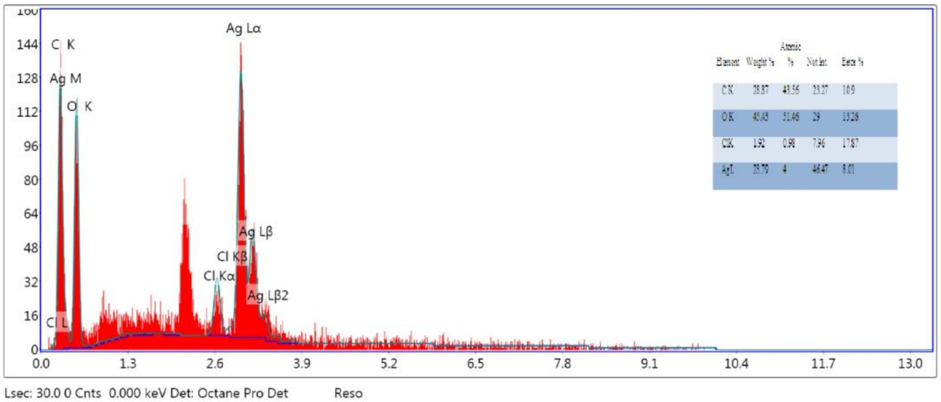


Figure 15: *EDXA analysis of treated viscose with AgNPs colloidal solution which prepared from a whole fruit (C. Colocynthis) at 100ppm concentration of silver nitrate*

Conclusion

We report the synthesis of silver nanoparticles by using *Citrullus Colocynthis* as redusing and stabilizing agent which not required special physical conduction. AgNPs were successfully synthesized under temperature 80^0C using different component of *C. Colocynthis* such as whole fruit, whole fruit without seeds and seeds only. The formation of AgNPs was confirmed in the uv-visible absorption spectra, which showed the characteristics band of AgNPs in the rage of 412-420. The TEM images showed that the AgNPs were homogenous and in spherical shape and the best one formed with the whole fruit. The application of AgNPs on viscose and wool fabrics in different concentration were confirmed via antibacterial properties and SEM ,EDXA, HPLC which confirm the formation of AgNPs with definite concentration .

Acknowledgement

The authors are gratefully acknowledged to the Prof. Dr. Abd -El Aziz Kantouch , Professor of Textile Chemistry and Technology for valuable support during the study period.

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