

MODIFICATION OF DATE PALM MUCILAGE AND EVALUATION OF THEIR NUTRACEUTICAL POTENTIAL

Muhammad Shahid¹, Fozia Anjum^{2,*}, Yasir Iqbal², Samreen Gul Khan² and Tajnees Pirzada³

¹Department of Biochemistry, University of Agriculture Faisalabad-38040, Pakistan; ²Department of Chemistry, Government College University, Faisalabad-Pakistan; ³Institute of Chemistry, Shah Abdul Latif University, Khairpur, Pakistan.

*Corresponding author's e-mail: drfoziaanjum@gcuf.edu.pk

In the present research project, *Phoenix dactylifera* mucilage was explored to disclose its modification and capping potential for encapsulation of silver nano particles. Process of silver nano formulation was monitored by running the sample on UV/VIS spectrophotometry. Antioxidant, antimicrobial activity as well as cytotoxicity of crude and modified mucilage were analyzed. Synthesized spherical nanoparticles were sized up to 39 nm. UV/ VIS spectrophotometric results revealed an intense peak at 427 nm along with other small peaks in region of 350-450 nm owing to the existence of poly disperse silver nanoparticles. Maximum phenolic contents were displayed by crude mucilage (230.37 ± 0.04 mg GAE/100g dw). Scavenging activity ($44.18 \pm 0.95\%$) and flavonoid contents (28.47 ± 0.07 mg QE/100g dw) were detected maximum in carboxymethylated mucilage. Nano formulated mucilage exhibited bactericidal activity maximum against *Escherichia coli* and minimum against *Staphylococcus aureus* and inhibition zone was calculated up to 35 and 17 mm respectively. Growth inhibition of *Fusarium solani* and *Aspergillus niger* by nano formulated mucilage was maximum (45.00 & 66.67%, respectively) of all the samples under investigations. The outcome exposed that such modified products might have potential applications in food as well as medicinal products.

Keywords: Date palm mucilage, nano particles, industrial products, antioxidant activity, antimicrobial activity

INTRODUCTION

In the current panorama of the industrial world, nano particles have become the blessing to solve many of the industrial problems such as in medicines, food, health related products and different industrial operations because of their physicochemical properties. These properties are related to their behavior in electrical field, behavior under heat treatment, response to different electromagnetic radiations and their biological properties etc. Among the nanoparticles, silver nano particles exhibited excellent behavior towards electrical, magnetic as well as health care properties (Zhang *et al.*, 2016; Bibi *et al.*, 2017).

As silver nano particles have excellent bactericidal effects and can retard the growth of bacteria as well as fungi. They heal up speedily wounds related to microbial infections. Due to their extraordinary medicinal effects, silver nano particles have tremendous application in medical devices, such as incorporated into bandages and medicines available commercially for their synergistic effects (Bibi *et al.*, 2017). Silver nano particles are produced by number of methods but biologically produced silver nano particles are more ecofriendly, qualitatively more compatible, easy to produce, more reliable to use and having more efficiency and dynamic character in the green nano world (Zhang *et al.*, 2016).

In the process of nano formulation, number of biomolecules is the key factors. Most of the plants parts or plant extracts act as capping agent/ green matrix in metallic nano formulation during the biosynthesis of nanoparticles. These biosynthesized nano particles are more effective, non-toxic, fast acting and involving less input. These simple and fast acting biosynthesis methods can be used for the production of nano particles commercially (Shahid *et al.*, 2016).

Physicochemical as well as biological methods are available for the nano particles formulation and synthesis. In comparison to chemical preparations, ecofriendly nano particles can be synthesized by using biological molecules that resulted in the production of non-toxic, energetic products. Chemical preparations of nano particles not only resulted in the formation of nano formulation but also emission of toxic chemicals that are threat to environment as reported by Khatami and Pourseyedi (2015).

In the past, for the production of metallic nano particles, different chemical or biochemical matrix (Starch and tannic acid) were used as capping material causing the reduction of metals. Recently, researchers have paid attention on the utilization of biological macromolecules such as carbohydrate polymers, gum and mucilages for the reduction of silver and gold during the process of nano formulation (Rashid *et al.*, 2016). Mucilage are present in most of the plants as a intracellular metabolite whereas gums are product

of plant produced due to abnormal attitude of the plant as a result of shock or damage to the plants due to environmental risks (Nazar *et al.*, 2017).

Fruit of *Phoenix dactylifera* is a rich source of mucilage. Fruits mostly consisted of carbohydrates and sugar of reducing nature. In addition, macromolecules of pectin, cellulose and starch are also present. Mucilages are mostly used in foods and medicine as an additives or binders. Binding properties of mucilage extracted from fruit of date palm mucilage has been reported previously. Nutraceuticals having mucilages of date palm as additives are less dusty than made from acacia and tragacanth. Binding ability, good consistency in weight and order are directly proportional to the mucilage or gum concentration (Bukhari *et al.*, 2014). Current research work has been made to extract mucilage from fruit of date palm. This mucilage was further subjected to modification as well as nano formulation and to evaluate their nutraceutical potential.

MATERIALS AND METHODS

Chemicals and reagents: For the whole experimental work, all chemicals and reagents of analytical grade, Merck, Sigma and Fluka brand were utilized. 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH), Folin-Ciocalteu reagent, and gallic acid were procured from Sigma Chemicals Co (St, Louis, MO, USA). All culture media and standard antibiotics were acquired from Oxoid Ltd. (Hampshire, UK). All other chemicals of analytical grade such as sodium carbonate, sodium nitrite, potassium dihydrogen phosphate and dipotassium hydrogen phosphate, used in the current study were purchased from Merck (Darmstadt, Germany), unless stated otherwise

Target sample for research: Mucilage of date palm (*Phoenix dactylifera*) was used for current research work that was purchased from Herbal medicine store, Karkhana bazar, Faisalabad, Pakistan.

Pretreatment: To free from dust and undesirable substances, mucilage sample was purified after washing and drying (Munir *et al.*, 2016). Mucilage dispersion was done in distilled water to homogenize in 24 hours that resulted in the formation of sticky solution. Solution was further treated with absolute ethanol that turned the solution into white creamy precipitates. This process was further repeated twice to purify the precipitates. The precipitates were dried then subjected to grinding by using mechanical blender and purified analytical sample was kept till further research work.

Carboxy methylation of mucilage: Carboxymethylation of mucilage was done by dispersion of 4.0 g purified mucilage in to 200 mL of distilled water that was followed by the incorporation of 20 mL of NaOH (5g/100 mL DW) and chloro acetic acid (7.0% v/v) at ambient temperature. Reaction turned the mixture into precipitates after the

introduction of ethanol. Precipitates of modified mucilage were made free from moisture at 50°C and incubated for further analysis (Munir *et al.*, 2016).

Nanoparticles formulation using mucilage as green matrix: Purified mucilage was dispersed in solvent by using magnetic stirrer (Gallen Kamp) for period of 360 min followed by the addition of 1 mM solution of silver nitrate. The mixture was sterilized for 15 min at 121°C and 15 psi. After sterilization, color of the mixture was changed which indicating the reduction of silver ions to silver nanoparticles. Mixture was centrifuged to separate the pellets that were re-dispersed in distilled water before freeze drying. Nano formulated particles were incubated safely in sterilized ampule that was covered with aluminum foil for further applications and characterization (Bibi *et al.*, 2017).

Analysis by using Zeta Sizer:

Particles, emulsions and suspended/ dissolved molecules can easily be characterized by using suitable technique such as Dynamic light scattering (DLS). Sample is exposed to a beam of laser that causing the scattering of light with different intensities. This scattered light is detected using detector. Particle size was calculated by Stokes-Einstein equation from scattered light after converting into diffusion coefficient. In the current research work, synthesized silver nano formulated particles were analyzed by using particle size analyzer (Malvern zeta seizer 2000, Malvern Instruments Ltd., U.K) at 24.9° with 90° detection angle (Jyoti *et al.*, 2016).

Characterization of crude, purified, modified and nano formulated date palm mucilage:

UV/VIS Spectrophotometric analysis: For identification and confirmation of nano formulation, qualitative analysis of mucilage having silver nano particles was done by using T60-UV-Visible spectrophotometer having light source of Deuterium lamp and tungsten/halogen lamp. Photodiode detector was used having wavelength scan in the range of 190-1100 nm. The spectra were recorded at the resolution of 1 nm as reported by Ata *et al.* (2018).

Biochemical analysis of crude mucilage:

Proximate composition: Moisture, protein, ash, fiber and fats analysis was performed by the standard methods of AOAC as reported by Shaba *et al.* (2015).

Antioxidant activity:

Determination of Diphenyl Picryl Hydrazile radical scavenging activity (DPPH): Mucilage was evaluated for antioxidant activity by measuring their efficiency to scavenge free radical of 2, 2-diphenyl-1-picryl-hydrazyl (Louaileche *et al.*, 2015).

Determination of phenolics: Phenolic contents of mucilage was determined by Folin-Ciocalteu method reported by Ainsworth and Gillespie (2007).

Determination of Total Flavonoids Contents (TFC): Flavonoids were analyzed by aluminum chloride

colorimetric method with slight modifications (Kamtekar *et al.*, 2014).

Antimicrobial activity: For the assessment of antimicrobial activity of mucilage sample, well diffusion method was adopted using different microbial strains.

Antibacterial activity: For the determination of antibacterial activity of mucilage under evaluation, *E. coli* (ATCC 35218) and *S. aureus* (ATCC 25923) strains were taken from the Department of Veterinary Microbiology, University of Agriculture, Faisalabad and subjected to purification and characterization.

(a) Medium and culture for bacterial growth: Petri plates were prepared with nutrient agar for bacterial growth. Furthermore, nutrient broth (13 g/L, Oxoid) was taken and subjected to sterilization for 15 min. Inoculation of pure culture of a bacterial strain was done with the help of loop followed by agitation in an orbital shaker at 37°C for 24 hours. Prepared inoculums was incubated at 4°C before running the assay for antibacterial activity (Saleem *et al.*, 2010).

(b) Antibacterial Assay by well diffusion method: In this method, bacterial strains such as *S. aureus* and *E. coli* were used to evaluate the antibacterial activity of mucilage samples. Nutrient agar (28 g/ 1000 L, Oxoid, UK) was autoclaved at 121°C for 15 min and settled at room temperature then followed by inoculation of microbes in this medium. Medium was transferred to sterilized petri plates to solidify. Afterwards, sample wells were made by using a sterile borer of 6 mm in diameter. Sample (50-70 µL) under investigation was taken in wells under sterilized conditions whereas Rifampicin was taken as positive control. Then plates were incubated for 24 hours in growth chamber under controlled conditions. Clear zones were appeared in those wells having samples that inhibited the growth of bacteria. Zone reader was used to measure these zones and standardized with *Rifampicin* (Balouiri *et al.*, 2016).

Antifungal activity: Samples under investigation were evaluated for their antifungal activity by using different fungal strains.

a) Growth medium and culture preparation for fungal strains: Potato dextrose agar (PDA) in sterilized petri plates was used to grow *A. niger* and *F. solani* and incubated for 96 hours at room temperature to multiply. After optimum growth of fungus, a loop full of fungus was introduced into sterilized media of sabouraud dextrose broth (SDB) (30g/L) then incubated at 28°C

for the period of 96 hours (Saleem *et al.*, 2010).

b) Antifungal assay by well diffusion method: In this method, sterilized petri plates were prepared by using PDA (39.0 g/ L) as a growth medium. Fungal inoculation was done in sample wells of 6 mm in diameter followed by loading of sample under investigation in comparison with standard antifungal agent that was *Terbinafine* (10 mg/mL) and incubated at 28°C for 48 hours. The fungal growth was inhibited with the appearance of clear zones in those wells having experimental samples with antiseptic behavior. These inhibited zones were further measured by using zone reader (Tariq *et al.*, 2014).

Toxicological study:

Hemolytic Assay: Analytical samples were evaluated for their *in vitro* hemolytic behavior (Powell *et al.*, 2000; Irshad *et al.*, 2017). After consent and counseling, heparinized human blood was collected from volunteers (3.0 mL) and centrifuged at 850 xg for five minutes and transferred into sterilized polystyrene tube of 15 mL capacity. Chilled phosphate buffer (pH 7.4) was used to isolate red blood cells from plasma. For each assay, 108 erythrocytes cells per mL were maintained in a tube of 2 ml capacity then followed by the addition of 180 µL diluted blood cell suspension and incubated for half an hour. Tubes were stabilized in ice for five minutes after agitation for ten minutes at 37°C then centrifuged at speed of 1310 xg and separated supernatant (100 µL) was diluted up 1000 µL with chilled phosphate buffer saline (PBS). Ice was used to stabilize all these tubes. 96 well plates were filled with 200 µL of this mixture. For maximum lysis, triton X-100 (0.1%) was taken as a positive control whereas phosphate buffer was taken as a negative control with exhibiting minimum lysis. Micro Quant Elisa Plate Reader (BioTek, Winooski, VT, USA) was used to make absorbance at 576 nm. All the protocol was repeated thrice for each experimental sample.

The RBCs lysis (%) was calculated by the following formula:

$$\text{RBCs lysis (\%)} = (\text{Absorbance of sample} - \text{Absorbance of Negative control}) / \text{Absorbance of Positive control} \times 100$$

Ames test for mutagenicity: Mutagenicity of experimental samples were evaluated by performing the Ames test by using Muta-Chromplate (EBPI, Ontario, Canada) Test was performed in liquid culture (Nutrient agar) of bacteria such as *S. typhimurium* TA98 and *S. typhimurium* TA100 (Ames *et al.*, 1975; Fluckiger-Isler and Kamber, 2014).

Table 1. Setup for Ames Assay.

Treatment (Micro well plate)	Sample extract	Standard mutagen	Reagent mixture	Deionized water	Test strain
Blank	----	----	2.5	17.5	----
Background	----	----	2.5	17.5	0.005
Standard mutagen	----	0.1	2.5	17.5	0.005
Test Sample	0.005	----	2.5	17.5	0.005

Procedure for Ames test: A reagent mixture of Davis-Mingioli salt, glucose-D, bromocresol purple, biotin-D and histidine-L was prepared in sterilized bottle. Test was performed according to the values as given in Table 1. Sample analyte was prepared by adding sample extract, reagent mixture, and test strain along with distilled water. In this test, micro well plates were prepared according to the method of Razak and Aidoo (2011) and incubated for 96 hours at 37°C. The reference plate was noted first followed by the experimental plates whereas wells having blank visualized colored (purple) indicating the non-contaminated assay. The background, standard and sample analyte were counted visually. Wells with the appearance of turbidity and yellow ting were counted as positive whereas wells having purple appearance were counted as negative. Purple coloration in the wells of sample analyte exhibiting the toxicity of sample against test strain. For an extract to be mutagenic, the number of positive wells had to be more than twice the number of positive well in the background plate.

Scheme of test: To perform Ames test, all chemicals and solution of test samples along with positive and negative standards were employed by following the scheme as mentioned in table 1. The volume of each mentioned in tables was added in mL.

RESULTS

Date palm mucilage obtained from dried fruit of *Phoenix dactylifera L.* is very commonly available on herbal and alternative medicine stores within Pakistan. It is dark brown in colour and in the form of pellets (Fig. 1a). It is highly viscous and insoluble in water so it might be an additive in food products.

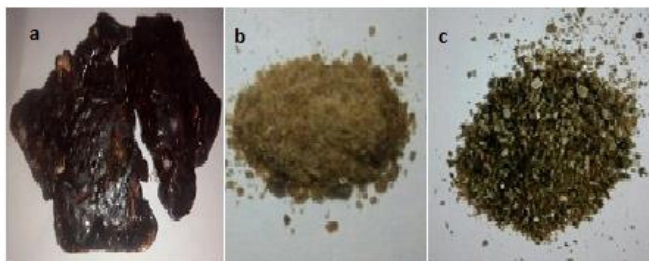


Figure 1. Mucilage a. Crude, b. Purified, c. Nano formulated.

Pretreatment and purification of raw sample: The mucilage obtained from local market was first made dust and dirt free and covered in polythene. For further research, mucilage was placed in distilled water for 24 hours. The sticky liquid smelling mainly glucose was obtained which was purified by using absolute ethanol.

The crude mucilage was subjected to purification. The percentage yield (73.75%) of the purified mucilage was

obtained by the method reported by Ameh (2013). After purification sample was turned into somewhat light brown powdered matter (Fig. 1b). The percentage yield is good and hence the mucilage could be used for many industrial applications as replacement of date fruit. The purified mucilage was further subjected to modification by carboxy methylation and yield (77.54%) of carboxy methylated mucilage as a new derivative.

Modification of mucilage by silver nanoparticles: In current study by using green synthesis, environmental friendly silver nanoparticles were synthesized successfully. The first visual and physical identification for the formation of nanoparticles is the change in color that has been observed due to the reduction of salt of metal to metallic nanoparticles. The solution containing metallic salt and mucilage after autoclaving at 121°C at 15 psi turned into dark brownish color which is the confirmation of the formation of silver nanoparticles (Fig. 1c).

Biochemical analysis of crude date palm mucilage: Crude date palm mucilage was subjected to proximate analysis and results are given in Table 2. It has been found that crude mucilage was enriched with fats (2.04%), proteins (2.19%), minerals (2.95%) and fiber contents (90.87%). Moisture contents were detected up to 7.68%. Carbohydrates of the crude date palm mucilage was found 85.16%, whereas total energy was calculated 367.83%.

Table 2. Proximate composition of crude mucilage.

<i>Phoenix dactylifera L.</i> mucilage (%)	Results
Crude fat	2.04±0.005
Moisture content	7.68±0.080
Crude protein	2.19±0.010
Ash content	2.95±0.050
Crude fiber	90.87±0.060
Total carbohydrates	85.16±0.036
Total energy (kcal/g)	367.83±0.140

Analysis of date palm mucilage by UV-Visible

spectroscopy: The biogenically based silver nanoparticles formed were spectroscopically analyzed and results are reported in Figure 2. Particles of nano formulation produced through efficient bio reduction of Ag⁺ to Ag⁰ showed an intense peak at 427 nm along with other small peaks in the region of 350-450 nm range (Fig. 2c). The sharp peak at 427 nm confirmed the formation of silver nanoparticles due to surface plasmon resonance. This was due to the presence of poly disperse silver nanoparticles. The sample gave peak at 139.7 nm with 100% intensity with average particle size of 166.4 nm. The UV-Visible spectra of purified and carboxymethylated mucilage are also given which show no peak in 400 nm region (Fig. 2a & b).

Antioxidative potential:

Activity to scavenge free radical: The potential of crude mucilage to scavenge free radical was detected 28.26 % that

become somewhat higher after purification (30.79%) and then modifications (44.18%). Maximum scavenging activity (47.12 ± 0.95) was shown by nanoformulated mucilage (Table 3).

Table 3. Antioxidant activities of mucilage samples.

Sample ID	DPPH scavenging (%)	Total phenolic (mg GAE/100g dw)	Total flavonoids (mg QE/100g dw)
Crude	28.26±0.54	230.37±0.04	22.21±0.06
Purified	30.79±0.38	122.41±0.08	25.56±0.07
Modified	44.18±0.95	43.14±0.03	28.47±0.07
AgNps	11.12±0.80	20.51±0.05	16.74±0.06

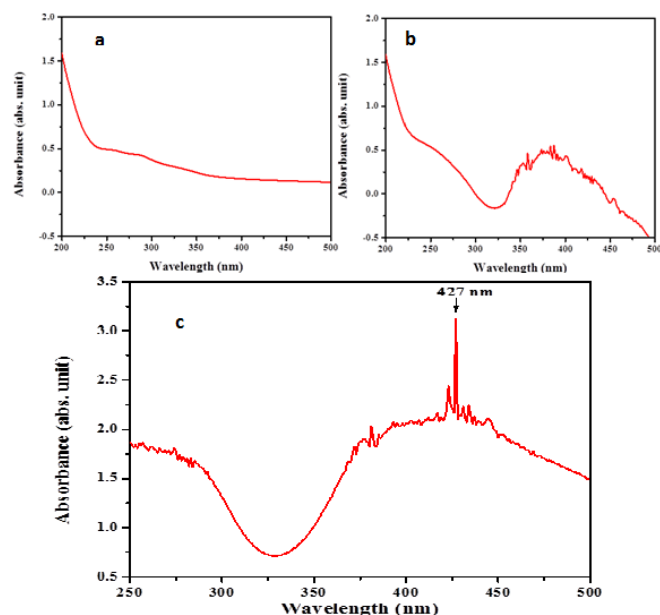


Figure 2. UV/ Vis spectra of mucilage samples; (a) Purified (b) Modified (c) Silver nanoparticles formulation.

Determination of phenolics: Phenolic contents were examined in all mucilage samples and results are reported in Table 3. Maximum value of phenolic contents was displayed by crude mucilage sample (230.37 ± 0.04 mg/ GAE/100g dw). This value decreased to 122.41 ± 0.08 when treated

with alcohol for purifications followed by the mucilage (43.14 ± 0.03 mg/ GAE/100g dw) undergo modifications with carboxy methylation. This value further reduced to its minimum (20.51 ± 0.05 mg/ GAE/100g dw) when sample was undergone nano formulations.

Total flavonoids contents: Total flavonoids were estimated in all experimental samples and results are reported in Table 3. It is clear from the table that flavonoids contents of 28.47 ± 0.07 mg QE/100g dw were found maximum in the sample of mucilage that was modified by carboxy methylations that might have increased the activity of flavonoids by the introduction of carboxyl group. Mucilage purified by alcoholic treatment displayed 25.56 ± 0.07 mg QE/100g dw flavonoids followed by crude mucilage (25.56 ± 0.07) whereas antioxidant activity due to flavonoids contents was reduced to 16.74 ± 0.06 in sample of mucilage that was subjected to nano formulations.

Antimicrobial activity:

Bactericidal activity: Bactericidal behavior of all experimental samples was determined by well diffusion method. Culture of control was treated with bactericidal Rifampicin and results are reported in Table 4. All experimental samples showed antibacterial activities against *E. coli* and *S. aureus*. Nano formulated mucilage exhibited bactericidal activity maximum against *E. coli* and minimum against *S. aureus* and inhibition zone was calculated up to 35 and 17 mm respectively. Mucilage that was purified by alcoholic treatment also showed significantly higher bactericidal activity against both bacterial strains and inhibition zone were measured as 32 and 36 mm respectively. Sample modified by carboxy methylation showed inhibition zone of 30 mm for both strains. Comparatively less antibacterial activity was found in crude mucilage that inhibited the growth of both bacterial strains and showed the zone of inhibition of 25 and 20 mm respectively.

Anti-fungal activity: Antifungal activity was performed against different fungal strains such as *F. solani* and *A. niger*. The results thus obtained are reported in Table 4. Growth inhibition by nano formulated and modified date palm mucilage was observed (45.00 & 66.67%) and (45.00 & 53.00%) followed by purified (35.00 & 46.67%) and crude mucilage (30.00 & 33.33%), respectively.

Table 4. Antimicrobial and hemolytic activities of mucilage samples.

Sample ID	Bacterial strains		Fungal strains		Hemolysis (%)
	<i>E.coli</i> (mm)	<i>S. aureus</i> (mm)	<i>F. solani</i> (mm)	<i>A. niger</i> (mm)	
Crude	25.0	20.0	6.0	5.0	1.80±0.04
Purified	32.0	37.0	7.0	7.0	3.21±0.01
Modified	30.0	30.0	9.0	8.0	5.89±0.12
AgNps	35.0	17.0	9.0	10.0	6.35±0.01
Positive control (<i>Rifampicin</i>)	48.0	48.0	-	-	-
Positive control (Terbinafine)	-	-	20.0	15.0	-
Positive control (Triton-X-100)	-	-	-	-	82.13±2.01

Table 5. Mutagenic activities of mucilage samples.

Sample ID	<i>S. typhimurium</i> strain TA 98		<i>S. typhimurium</i> strain TA 100	
	No. of positive wells/total wells	Result	No. of positive wells/total wells	Result
Blank	-	Not contaminated	-	Not contaminated
Background	7/96	-	10/96	-
Standard	75/96	Mutagenic	72/96	Mutagenic
Crude	1/96	Non-mutagenic	1/96	Non-mutagenic
Purified	7/96	Non-mutagenic	1/96	Non-mutagenic
Modified	6/96	Non-mutagenic	3/96	Non-mutagenic
AgNps	1/96	Non-mutagenic	2/96	Non-mutagenic

Toxicological and mutagenicity study:

Hemolytic assay: In the present research work, crude, purified, modified and silver nanoparticles were evaluated for hemolysis. The fresh human blood from volunteers was used to evaluate the percentage hemolysis of samples. All the samples showed somewhat little non-significant hemolysis (Table 4). Hemolytic activity of crude mucilage was detected up to 2.0%. Hemolytic activity was increased as the mucilage was processed for purification (3.90%), modification (7.17 %) and then nano formulation (7.73%).

Mutagenicity assay by Ames test: The samples were tested against two bacterial strains *Salmonella typhimurium* strain TA 98 (Table 5) and *Salmonella typhimurium* strain TA 100 (Table 5) for the possible mutagenic activity. For current research, all the samples under evaluation were non-mutagenic.

DISCUSSION

Phoenix dactylifera is a rich source of mucilaginous material that is obtained from dried fruit in the form of pellets. In the current research, viscous mucilage was obtained that was dark brown in color and have no solubility in water.

Pretreatment and purification of raw sample: Mucilage was subjected to pretreatment after collecting from the market. Sample was treated with water to remove dust and any dirty particles attached to it. Mucilage was then treated with absolute ethanol and converted into somewhat gummy material having odour that might be due to the formation of glucose as a result of ethanolic treatment (Ameh, 2013). This gummy material was turned into light brown color after drying and modification.

Biochemical analysis of crude date palm mucilage: Mucilage was processed for biochemical analysis and results are depicted in Table 2. It is clear from the table that crude mucilage has potential dietary value due to presence of fats, proteins, minerals and fiber contents. In addition, carbohydrates and total energy was also analyzed and have results more significant as compared to the findings of different mucilage reported by Rodriguez-Gonzalez *et al.* (2014). They reviewed the mucilage collected from dried fruit of date palm that contained significant amount of

reducing sugars in carbohydrates in addition to proximate analysis. Similarly, Jamil *et al.* (2010) studied the proximate composition and mineral analysis of unexplored diverse varieties of date palm collected from different geographical regions of Pakistan. In their research findings, moisture contents analyzed were from 1.6 to 9.8% whereas inorganic minerals were from 1.82- 2.87%. In their study, contents of protein (32.5-41.25%) were highly significant as compared to present research work data and crude fiber was noticed in the range of 62.11 to 86.08% that is significantly lower as compared to present findings. It is assumed that gums and mucilage's have more amounts of crude fibers than seed or fruit.

Elleuch *et al.* (2008) performed research work on discarded dates of two varieties named Deglet-Nour and Allig and analyzed their chemical composition. They reported the protein 2.1 and 2.5%, and ash contents 3.00 and 2.52%, respectively. These findings are in close agreement with the present research outcomes.

Silver nanoparticles formulation using mucilage as green matrix: Silver nanoparticles were synthesized by using green synthesis pathway using date palm mucilage as green matrix. As green matrix has capping and reducing potential for the synthesis of nanoparticles due to the presence of phenolics, flavonoids and carbohydrates having reducing sugars in any part of plant. Nano particles having green matrix as capping material are more reliable, easy to make and having no toxicity. In the present research area, ecofriendly silver nano formulation has been done successfully by using date palm mucilage as green matrix that was visualized and identified physically by change in color as a result of bio reduction of metallic salt into metallic nanoparticles. Color change from brownish to dark brownish purple coloration is the confirmation of nano particles as a result of heat treatment (Fig. 2c). During synthesis of nano formulation, change in color might be due to the reduction of metallic salt by using green capping material. In consistent to our result, Sun *et al.* (2014), Ajitha *et al.* (2015), Rashid *et al.* (2016), Jayaprakash *et al.* (2017) and Farhadi *et al.* (2017) also examined the silver nano particles formulation and alteration in color to yellowish brown. In another research study, coloration of silver nano particles

was developed from colorless to dark brown color as reported by Prakash *et al.* (2013). Similarly, Aitneite *et al.* (2016) examined the change in coloration from brown to deep red as the confirmation of silver nanoformulation.

Analysis of date palm mucilage by UV-Visible spectroscopy: Environmentally compatible nano formulations of silver have been formed by adding the mucilage solution into silver nitrate under heat treatment for specific period of time (Fig. 2). Shape and size of nano particles are dependent on heat treatment as reported by Kora *et al.* (2010) and in the present research work same trend in shape and size of nanoparticles of silver has been observed and has been proven experimentally that mono dispersion of nano particles are directly proportional to the increase in heating time of nano particles formulation thus more stable and smaller size of the nano particles produced than in previous research work (Sosnowska, 2009; Kora *et al.*, 2010; Jayaprakash *et al.*, 2015). The confirmation of silver nano particles were then confirmed by spectrophotometry by taking absorption peak at 427 nm that might be due to surface plasmon resonance and presence of poly dispersed nano particles are the main reason for surface plasmon resonance. In addition, a peak having 100% intensity was appeared at 139.7 nm of particle size of 166.4 nm. There might be anisotropic behavior of synthesized nano particles as state by Mie's theory "Single absorption peak appear from spherical nanoparticles of silver that will be in ordered form while appearance of two or more absorption bands in place of absorption peak corresponds to the allotropic form of silver nanoparticles (Jayaprakash *et al.*, 2015). In comparison of absorption spectra of nano formulated mucilage, pure as well as modified mucilage has given no absorption peak up to 400 nm region. Aitneite *et al.* (2016) and Farhadi *et al.* (2017) also reported the prominent peak at 420 and 415nm of silver nanoparticles of leaf extract of date palm that might be due to the excitation of surface plasmon vibrations. In the earlier study silver nanoparticles were detected having maximum wavelength values in the range of 400-500nm (Singhal *et al.*, 2011; Johnson *et al.*, 2014).

Antioxidant activity: Auto oxidation and its different steps like initiation or propagation can be inhibited or delayed by using organic compound having antioxidant activity that are present in naturally occurring resources of biomolecules. These biomolecules are rich source of bioactives such as phenolic, flavonoids, terpenoids that have antioxidative behavior. These bioactives exhibited antioxidative activity might be due to their oxidation/ reduction properties. Redox properties of bioactives are responsible for the absorption and neutralization of free radical thus singlet/ triplet oxygen are quenched and peroxides are decomposed (Javanmardi *et al.*, 2003). Similarly Bokhari, and Perveen (2012) analyzed the presence steroidal compounds, alkaloids, carbohydrates flavonoids, tannins and saponins etc. in different parts of *P.*

dactylifera mucilage modified by carboxy methylation exhibited oxygen quenching activity maximum whereas nano formulated mucilage quenched minimum of oxygen from oxidative product that might be due to decomposition of antioxidants such as flavonoids as a result of nano particles formulations. Trend of the scavenging activity of mucilage is consistent with the presence of flavonoids in these samples. As flavonoids are maximum in mucilage modified with carboxy methylation. Incorporation of carboxyl group in mucilage has synergistic effects on scavenging of oxygen and thus enhance the efficiency of flavonoid to attack on oxidative products such as peroxides or hydroperoxides. Likewise, Xu *et al.* (2009) performed carboxy methylation of polysaccharides that was separated out from *Ganoderma lucidum*. They found the same results related to antioxidant activity of modified polysaccharides. Similarly biopolymers modified by carboxy methylation exhibited antioxidant behavior and scavenge oxides, peroxides and superoxides. These present results and their trends are in close agreement with the previously reported results (Kamtekar *et al.*, 2014; Louaileche *et al.*, 2015).

Most of the clinical disorders such as heart disease, cancerous diseases, diabetes etc. are due to presence of free radical that causing the oxidative stress. These free radicals are singlet electron and are produced as a result of any heat shock or by irradiation. Free radicals are highly reactive and causing initiation of chain reactions. As a result of chain reaction, cell organelles, like lipo-proteins, vitamins, nucleic acid undergo decomposition. Different body systems such as catalase, super oxide dismutase (act as terminators) offer protective actions against chain reactions created by free radicals. In serious cases of damage of cell organelle due to chain reaction of reactive oxygen species, antioxidant supplements are prescribed (Al-Mamary *et al.*, 2014).

Activity to scavenge free radical: In the current research work, stable free radical of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was taken to evaluate the potential of mucilage sample to scavenge it. As anti free radical activity exhibited by those compounds that have higher reducing power. This DPPH free radical show absorption maximum at 517nm after the acceptance of electron from nucleophile. In the current research work, intensity of absorption at 517nm was decreased as research samples are not electron donor to DPPH radical (Table 3). Maximum scavenging activity was detected in nanoformulated sample (47.12%), followed by modified (44.18%), purified (30.79%) and crude sample (28.26%). Maximum scavenging activity by nanoformulation might be due to the development of spherical and small sized particles that not only increased the exposed surface area but also efficiency to scavenge free radicals hence they will increase the efficiency of the body's immune system which allowing the system to compete with pathogenic microbes that attack the body. Present research findings are in close agreement with the research data

reported by Gao *et al.* (2002), Tahir *et al.* (2015) and Tahir *et al.* (2016).

Determination of phenolics: Plants produce number of potentially important compounds as a result of metabolism or metabolic activities. These compounds or metabolites are phenolics, polyphenolics, flavonoids, terpenoids, triterpenoids, tocopherols, tocotrienols etc. All these metabolites are potential antioxidants and inhibit the lipid oxidation of lipoprotein due to the presence of hydroxyl group having acidic characters. Major class of phenolics includes cyanins, anthocyanins, cinnamates, tannins and flavonoids that constitute the major part of the organic carbon present in biosphere. These phenolics play an important role in quenching the reactive oxygen radical preferably than electron reduction (Ainsworth and Gillespie, 2007).

In the present research samples, phenolics were detected in a significant amount and results are reported in Table 3. In consistent to our results, Saleh *et al.* (2011) also reported the phenolics 238.54 mg/100 g in distilled water in ripened fruits of date palm (khalas variety) whereas phenolics of distilled water extract was also detected by Louaileche *et al.* (2015). These results reported previously are in accordance with the findings as detected in present results.

Total flavonoids contents: Total flavonoid contents were also detected in all mucilage samples and maximum flavonoids were detected in carboxy methylated mucilage that might have increased antioxidant activity of flavonoids by the introduction of carboxyl group (Table 3). The present research results have close proximity with the findings reported by Wang *et al.* (2015). They evaluated that incorporation of carboxyl group into mucilage structure might affect the bioactive character of bioactive compounds such as proteins, lipids, uronic acids and carbohydrates. As flavonoids are potential antioxidants and bioleach metals or chelate metals or metalloids. They are also enemy of reactive oxygen species or singlet oxygen and engulf/quench oxygen from auto oxidation process or oxidation chain reaction (Nevas *et al.*, 2004).

Antimicrobial activity: Customers are more conscious about their health now than before. To maintain their health they always ready to spend more. Nowadays there is increasing trend to use natural products having antioxidant and antiseptic characters as compared to synthetic products. As synthetic products have more antiseptic character but also have much more side effects. In the last decade, synthetic antimicrobials or antiseptic products have become useless as microbes or pathogens have evolved more resistant. Therefore, there is need of time to explore better substitute for eradication of microbes as antiseptic in the form of new medicine or antibiotics. Natural resources are the best sources of antiseptic formulations that can be more effective and having least toxicity. Up to 300 bioactive compounds extracted from natural resources have been reported in the

last eight years. These compound evaluated for their antiseptic character (Saleem *et al.*, 2010).

Antibacterial activity: In the present research work, mucilage samples showed antibacterial activity and present research findings are in accordance with the findings as reported by Ravishanker and Raut (2016). In their study they observed that date palm bark methanol fraction exhibited excellent activity against microbial strains with zones of inhibition of 22.0 mm against *S. aureus* and 20.0 mm against *E. coli*. Farhadi *et al.* (2017) reported the antibacterial effects of nanoformulated date palm fruit extracts and inhibition zones of 13.0 and 11.0mm were measured against *S. aureus* and *E. coli*. Recently many studies have been reported regarding the outstanding bactericidal effects of silver nanoparticles (Sun *et al.*, 2014; Ajitha *et al.*, 2015; Khatami and Pourseyedi, 2015; Rashid *et al.*, 2016). Jayaprakash *et al.* (2017) evaluated the bactericidal effects of synthesized silver nanoparticles, precursor salt AgNO₃ and fruit extract against eight bacterial strains and concluded that synthesized silver nanoparticles showed better antibacterial activities against all strains than silver nitrate and fruit extract that might be due to the existence of polyphenols in its structural composition. In consistent to our results, Tahir *et al.* (2016) reported the antibacterial potential of date palm mucilage based nanoparticles. These nanoparticles exhibited strong antibacterial efficiency against *pseudomonas aeruginosa* 26 (±0.8 mm). This high activity of PdNPs might be due to their small size, high dispersion and surface capping phytochemicals

Anti-fungal activity: Mucilage samples were evaluated for their antifungal behavior and found positive results against *F. solani* and *A. niger*. Similar results have been reported by Boulenouar *et al.* (2011). They evaluated the antifungal effects of dichloromethan and ethyl acetate extracts of eight date palm cultivators and found the maximum antifungal activity against *Fusarium oxysporum* 32.50%. Results of antimicrobial activity was reported by Khatami and Pourseyedi (2015). They found that date palm pit aqueous extract based silver nanoparticles inhibited the growth of *Rhizoctonia solani* and observed that a concentration of 25µg/mL of silver nanoparticles almost inhibited the growth of 83.00% of fungus mycelium.

Toxicological and mutagenicity study:

Hemolytic assay: Present research samples exhibited hemolysis to some extent but not significantly incorporated in to samples by processing. Similar results are reported by Shahid *et al.* (2013) who analyzed the little hemolytic activity of grafted guar gum sample up to 2.70% whereas crude sample exhibited no hemolytic activity for human blood sample. In the current research findings, values are in range and these samples could be used in pharmaceutical formulations. Further studies could be designed to improve the current results.

Mutagenicity assay by Ames test: For the determination of mutagenicity and carcinogenicity, Ames assay for reverse mutagenicity was used as developed by Ames *et al.* (1975) but the test was performed entirely on liquid cultures known as liquid fluctuation test (Razak and Aidoo, 2011). The validity of test is based on original Ames assay. For current research, all the samples under evaluation were non-mutagenic. Similar results are reported by Des *et al.* (2011) and Rashid *net al.* (2016). These results enhanced the efficacy of formulations for medicinal uses.

Conclusion: It could be concluded that *Phoenix dactylifera* mucilage has antioxidative as well as medicinal potential. Crude and purified mucilage has capping potential for encapsulation of silver nano particles. Nano formulated mucilage exhibited pronounced effects against bacteria and fungi and these modified products have number of applications in different food stuffs.

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