

Nanoparticles of freeze-dried *Garcinia mangostana* L. peels and its effective on the protein formation of Gram positive bacteria

Hany Mohamed YEHIA^{1,2*}, Manal Ahmed Gasmelseed AWAD³, Manal Fawzy ELKHADRAGY⁴,
Mariam Abdulaziz ALKHATEEB⁴, Hadil ALAHDAL⁴, Hatem Salama Mohamed ALI¹, Reem Atta ALAJAMI⁵,
Mohamed Ferkry Serag El-Din^{1,6}

Abstract

A novel method to create nanoparticles from freeze- and air-dried mangosteen was developed to study the effects of these particles on the growth and protein formation of various gram-positive bacteria that act as foodborne pathogens. This new method produces freeze- and air-dried mangosteen peel nanoparticles that are prepared by a process based on a wet-milling technique, and these particles were tested on various gram-positive pathogenic bacteria. Our results indicated that the nanoparticles derived from freeze dried mangosteen contained higher antioxidant activity than nanoparticles derived from air-dried peels. The total phenol content in the freeze-dried nanoparticle extract was 1112.646 ± 1.842 (mg gallic acid /g sample), whereas that in the air-dried extract was 479.744 ± 2.564 (mg gallic acid/g sample). The total flavonoids in the mangosteen freeze-dried nanoparticle extract were 14.154 ± 0.119 (mg catechin/g sample), whereas levels in the air-dried extract were 4.711 ± 0.207 (mg catechin/g sample). The levels of 2,2-diphenyl-1-picrylhydrazyl (DPPH) were 95.707 ± 0.070 and $94.303 \pm 0.074\%$ for freeze- and air-dried mangosteen nanoparticle extracts, respectively. Similar levels were obtained for 2,4,6-tri (2-pyridyl)-s-triazine (ABTS), and these were 42.753 ± 0.200 and 16.069 ± 0.424 (g trolox/g sample) for freeze- and air-dried mangosteen nanoparticle extracts, respectively. Levels of ferric reducing antioxidant power (FRAP) were 17.806 ± 0.056 and 6.696 ± 0.085 (g trolox/g sample) for freeze- and air-dried mangosteen nanoparticle extracts, respectively. Whole protein bands from various bacteria disappeared on SDS-polyacrylamide gels when bacteria were cultured in medium containing both freeze- and air-dried mangosteen nanoparticles.

Keywords: freeze dried mangosteen nanoparticles; antioxidants; proteins; SDS-polyacrylamide gel; G+ bacteria.

Practical Application: Nanoparticles of freeze-dried *Garcinia mangostana* L. peels is more effective than air dried on the protein formation of Gram positive bacteria.

1 Introduction

The queen fruit is named “Mangosteen” (*Garcinia mangostana* L.) because it is considered to be one of the best tasting tropical fruits. The milky white portion of the mangosteen is the edible portion, whereas the peel is dark red and is approximately twice the mass of the edible portion (Fu et al., 2007; Zarena & Udaya Sankar, 2012). A number of researchers have found bioactive compounds within the mangosteen peel that may be useful as potential functional food additives and therapeutic agents, and these include phenolic acids (Zadernowski et al., 2009), tannins (Pothitirat et al., 2009), xanthones (Zarena & Sankar, 2009), anthocyanins (Palapol et al., 2009), and other bioactive compounds. The biological effects of compounds isolated from the mangosteen peel include antimicrobial (Suksamrarn et al., 2002), antioxidant (Jung et al., 2006), and anti-inflammatory (Chen et al., 2008) effects.

New antibacterial drugs were extracted from natural products, so from here came the importance of such plants (Dkhil et al., 2016). Mangosteen is considered to be (*Garcinia mangostana*) as a one of the most desirable tropical fruits of Southeast Asia (Nazre, 2014). The mangosteen plant is distributed mainly in Indonesia, Malaysia, the Philippines, and Thailand (Gutierrez-Orozco & Failla, 2013), and contains secondary metabolites such as flavonoids and polyphenols. Mangosteen contains a rich source of polyphenols known as xanthones (Ayman et al., 2019). It has many benefits as antitumor, antifungal, antibacterial, anti-inflammatory and antioxidants so, it is a promise for pharmacological, medicinal, and cosmetic applications (Rajakannu et al., 2015).

Silver nanoparticles used in the manufacture of plants has drawn attention because its ecofriendly, nonpathogenic, simple and rapid. Through biosynthetic process of these nanoparticles it is provide large quantities of product in a single-step technique.

Received 02 Dec., 2021

Accepted 08 Jan., 2022

¹Food Science and Nutrition, College of Food and Agriculture Science, King Saud University, Riyadh, Saudi Arabia

²Department of Food Science and Nutrition, Faculty of Home Economics, Helwan University, Egypt

³King Abdullah Institute for Nanotechnology, King Saud University, Riyadh, Saudi Arabia

⁴Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

⁵Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

⁶Department of Food Science and Nutrition, Faculty of Home Economics, Minufiya University, Egypt

*Corresponding author: hanyehia@ksu.edu.sa

The medicinal values of silver ions reduction and stabilization by combining them with biomolecules, such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, and vitamins, which already exist in the plant extracts, have medicinal value (Tsai et al., 2016). There is increasing interest in the using of nanotechnology for biomedical purposes could form the bulk of future treatment strategies for different diseases (El-Khadragy et al., 2018). Furthermore, Allahverdiyev et al. (2011), found that Ag-NPs have anti-inflammatory effects by inhibiting metabolic activity through impairing mitochondrial function via oxidative stress.

The quality of the mangosteen peel extract quality is dependent upon its antioxidative performance as well as the environmental and technological factors affecting these activities. Thus, our study utilized a nanoparticle-based technique to obtain extracts from freeze- and air-dried mangosteen to examine the antioxidant properties of these extracts and to test their antimicrobial activity against gram-positive pathogenic bacteria.

2 Materials and methods

2.1 Sample preparation

Preparation of mangosteen peel

Healthy and fresh *Garcinia mangostana* fruit was collected from the hypermarket at Riyadh City, Saudi Arabia, and carefully washed with deionized water several times to remove dust particles. The fruit was then separated to two parts: one part was air-dried to remove the residual moisture, cut into small pieces, and stored in air-tight container. The second part was cut into small pieces, loaded onto a tray, and freeze-dried on a shelf in a freeze dryer (Labconco 8811 Prospect Ave, Kansas City, MO 64132, USA). This was then ground to a powder for further extractions.

Preparation of nanoparticles from mangosteen peel

Air- or freeze-dried mangosteen peel (400 mg) was dissolved in methanol (20 mL), and this solution was sprayed dropwise into boiling water (50 mL) at a flow rate of 0.2 mL/min for 5 min under ultrasonic conditions, where ultrasonic power was 750 W and the frequency was 20 kHz. After sonication for 20 min, the contents were stirred at 200–800 rpm at room temperature (20 °C) for approximately 15 min. The solution was then freeze-dried to obtain mangosteen nanoparticles.

2.2 Methods for determining antioxidant activity of mangosteen peel extract:

Total phenolic compounds

The content of total phenolic compounds in the methanol extracts was determined by the Folin–Ciocalteu method as described by Wu et al. (2007). A volume of 2.5 mL of distilled water and 0.1 mL of a sample extract were both added to a test tube, and this was followed by the addition of 0.1 mL of undiluted commercially available Folin–Ciocalteu reagent (Sigma-Aldrich,

St. Louis, MO, USA). The solution was mixed thoroughly and then allowed to stand for 6 min before 0.5 mL of a 20% sodium carbonate solution was added. Color developed after 30 min at room temperature, and the absorbance was measured at 760 nm using a spectrophotometer (Milton Roy Spectronic 1201, USA). A blank sample was prepared using 0.1 mL of methanol instead of extract. The measurement was compared to a calibration curve generated by gallic acid solutions, and values were expressed as gallic acid equivalents per gram of dry weight sample.

Total flavonoid content

The total flavonoid content of crude extract was determined by the aluminum chloride colorimetric method described by Baba & Malik (2015). Briefly, 50 µL of crude extract was mixed with 4 mL of distilled water and thereafter, with 0.3 mL of 5% NaNO₂ solution. A 0.3 mL volume of 10% AlCl₃ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Next, 2 mL of a 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL using distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight or mg catechin equivalent per g dry weight.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The ability of the samples to scavenge DPPH radicals was determined according to the method of Akillioglu & Karakaya (2010). A 0.08 mM DPPH radical solution was prepared in methanol, and 950 µL of DPPH stock solution was added to the 50 µL extract, followed by incubation for 5 min. Exactly 5 min later, absorbance readings of mixture were performed at 515 nm (Cary 50 Scan; Varian). The antioxidant activity (AA) was expressed as percent inhibition of DPPH radical using the equation $AA = 100 - [100 \times (A_{\text{sample}} / A_{\text{control}})]$; where, A_{sample} is the absorbance of the sample at t=5 min and A_{control} is the absorbance of the control.

ABTS (2,4,6-Tri(2-Pyridyl)-s-triazine) radical scavenging activity

The ABTS^{•+} assay was performed according to the method described by Gouveia & Castilho (2011). The ABTS^{•+} radical solution was prepared by mixing 50 mL of 2 mM ABTS solution with 200 µL of 70 mM potassium persulfate solution. This mixture was stored in the dark for 16 h at room temperature, and it was maintained in this form for two days. For each analysis, the ABTS^{•+} solution was diluted with pH 7.4 phosphate buffered saline (PBS) solution to an initial absorbance of 0.700 ± 0.021 at 734 nm. This solution was prepared fresh for each set of analyses. To determine the antiradical scavenging activity, an aliquot of 100 µL methanolic solution was added to 1.8 mL of ABTS^{•+} solution, and the absorbance decrease at 734 nm was recorded over a period of 6 min. The results were expressed as µmol trolox equivalent per g of dried sample (mmol eq. trolox/g) based on the trolox calibration curve.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay was performed according to the procedure described by Benzie and Strain (1996). The FRAP reagent included 300 mM acetate buffer at pH 3.6 and 10 mM tripyridyl triazine (TPTZ) in 40 mM HCl/20 mM FeCl₃ at a ratio of 10:1:1 (v/v/v). FRAP reagent (3 mL) was mixed with 100 µL of the sample extract in a test tube and vortexed in the incubator at 37 °C for 30 min in a water bath. Reduction of ferric-tripyridyltriazine to the ferrous complex resulted in the formation of an intense blue color, which was measured using a UV–vis spectrophotometer (Varian Cary 50) at 593 nm after 4 min. The results were expressed as mmol trolox equivalent per g of dried sample (mmol eq. trolox/g).

2.3 Antimicrobial assays

Gram-positive bacteria

Staphylococcus aureus ATCC 29737, *Listeria monocytogenes* ATCC 19114, *Bacillus cereus* ATCC 11778, *Micrococcus luteus* (local isolate), and *Enterococcus faecium* (local isolate) from laboratory of Food Microbiology, College of Food and Agriculture Sciences, King Saud University were used in this study.

Disc diffusion method

The disk diffusion method, described by Bauer et al. (1966), is a standardized technique for testing rapidly growing pathogens. Briefly, a standardized inoculum (100 µL of overnight culture of any bacteria tested that contained ~10⁶/mL) was swabbed onto the surface of Muller Hin agar (Oxoid, CM0337, 150-mm plate diameter). Filter paper disks impregnated with a standardized concentration of 100 µL (100 mg/mL) of either freeze- (FN) and air-dried (AN) mangosteen peel nanoparticles were placed onto the surface and compared to normal freeze- and air-dried mangosteen peel extracts (F and A, respectively). Discs saturated with methanol were used as a control (C). The size of the zone of inhibition surrounding the disk was measured after overnight incubation at 37 °C for 24 h.

Cell extracts preparation

According to a modification of the method described by Yehia & Al-Dagal (2014), an overnight culture (100 µL) was inoculated into a 10-mL volume of fresh medium (brain heart infusion, Oxoid, CM1135) supplemented with methanol (1 mL) as control or brain heart infusion medium, containing either air- and freeze-dried mangosteen peel methanol extracts, that was sterilized by filtration through a 0.45 µm Millipore filter. All strains of bacteria were grown at 37 °C for 24 h. Cells were then collected and weighed, and 250 mg of cells were then suspended in 100 µL of TES buffer (50 mM tris HCl, pH 8, 1 mM EDTA, 25% sucrose). Twenty microliters of lysozyme (50 mg/mL) was added to the suspended cells in the TES buffer and incubated at 37 °C for 30 min. Five to ten microliters of 20% SDS was then added, and the contents were mixed until the cells were clearly visible. The contents were stored at –20 °C until use. Twenty-five microliters of the extracts (bacteria in normal medium with

ethanol, bacteria with air-dried mangosteen peel nanoparticles, or bacteria with freeze-dried of mangosteen nanoparticles) specific to each of the five strains of bacteria tested was loaded onto SDS-PAGE. Electrophoresis was performed at 25 °C in a vertical tank apparatus using a constant voltage power supply until a bromophenol blue tracking dye reached the bottom of the gel. Gels were stained with 0.25% Coomassie brilliant blue R-250 (Bio-Rad, Marnesla-Coquette, France) in water:methanol:acetic acid (6.5:2.5:1) for 18 h at room temperature. Gel destaining was performed by continuous agitation in a methanol:acetic acid:water (20:10:70 v/v/v) solvent until obvious bands of proteins were obtained. Whole-cell protein profiles of tested bacteria were compared to air- and freeze-dried mangosteen nanoparticles, and the formation of protein bands was observed following SDS-PAGE.

3 Results and discussion

3.1 Characterization of mangosteen nanoparticles

Dynamic Light Scattering (DLS) analysis using Zetasizer

DLS is primarily used to obtain the average diameter of nanoparticle size and to determine the particle size distribution within solutions. The DLS diagram of the mangosteen peel freeze- and air-dried nanoparticles is presented in Figures 1 and 2, respectively. The results revealed that the average size of the freeze-dried mangosteen peel particle diameter was 123.1 nm with a polydispersity (PDI) of 0.382 and similar sizes (Figure 1). The average size of the air-dried mangosteen peel particle diameter was 153.1 nm with a polydispersity (PDI) of 0.241 and diverse sizes (Figure 2).

A zeta value of ± 30 mV is needed for a suspension to be physically stable while ± 20 mV is necessary for a combined electrostatic and steric condition (Faried et al., 2016). The zeta potential results for pure *G. Mangostana* peel extract are –14.68 mV, whereas the reading of Au-NPs formed using the extract reduced to –20.82mV. Thus, Au-NPs formed show an acceptable stability with reading not less than the required stable expression (Lee et al., 2016).

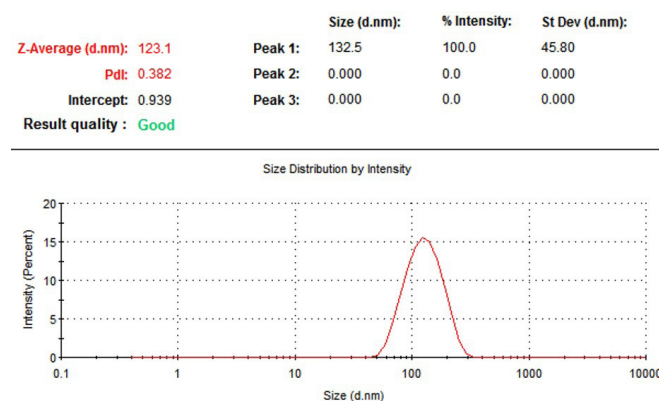


Figure 1. Zetasizer measurement of freeze-dried mangosteen peel nanoparticle size.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was used to obtain essential information regarding primary nanoparticle sizes and morphologies (Figures 3, 4). The freeze-dried mangosteen peel nanoparticles examined by TEM exhibited spherical and rod-shaped morphology (Figure 3). Additionally, all the resulting nanoparticles were similar in size. The air-dried mangosteen peel nanoparticles investigated by TEM exhibited only rod-shaped morphology (Figure 3), and the particles were of various sizes (Figure 4). Lee et al., 2016, determined the average size of peel extract of plant, *Garcinia mangostana* (*G. mangostana*) which synthesized and was 32.96 ± 5.25 nm with mostly spherical and some hexagonal and triangular shape.

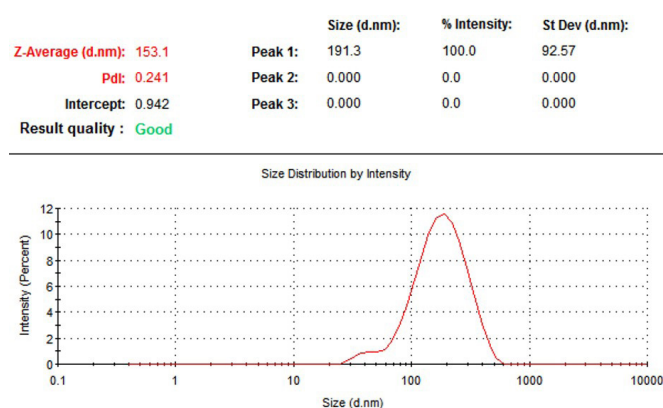


Figure 2. Zetasizer measurement of air-dried mangosteen peel nanoparticle size.

Antioxidant activity of mangosteen peel nanoparticles

Data in Table 1 indicate the total phenols that were determined in the methanol-based extraction of freeze-dried mangosteen peel nanoparticles. This value was determined to be 1112.646 ± 1.842 mg gallic acid /g sample. This quantity was larger than that found in normal freeze-dried mangosteen peel, which were 815.311 ± 3.935 mg gallic acid /g sample. The total phenols in the air-dried mangosteen nanoparticles did not exceed 479.744 ± 2.564 mg gallic acid /g sample. The total flavonoid content in the freeze-dried mangosteen peel nanoparticles and the normal extracts was almost equal, and both values were greater than that found in the air-dried mangosteen peel nanoparticles. The antioxidant activity (i.e., free-radical scavenging (DPPH)) of freeze-dried mangosteen peel nanoparticles was $95.707 \pm 0.070\%$, whereas this value in air-dried mangosteen nanoparticles was $94.303 \pm 0.074\%$. The ABTS value in freeze-dried mangosteen nanoparticles was 42.753 ± 0.200 (g trolox/g sample), whereas this value in air-dried mangosteen peel nanoparticles was 16.069 ± 0.424 (g trolox/g sample). The ferric reducing antioxidant power (FRAP) of freeze-dried mangosteen peel nanoparticles was 17.806 ± 0.056 (g trolox/g sample), whereas this value in air-dried mangosteen peel nanoparticles was 6.696 ± 0.085 (g trolox/g sample). The antioxidant activity for almost all freeze-dried mangosteen peel nanoparticles was higher than that determined for air-dried mangosteen peel nanoparticles. This result was expected, given the observed antimicrobial activity of the freeze-dried mangosteen peel nanoparticles.

Antibacterial activity of mangosteen nanoparticle against Gram positive bacteria

The results presented in Figure 5 clearly detail the effect of mangosteen peel nanoparticles on the growth of bacteria.

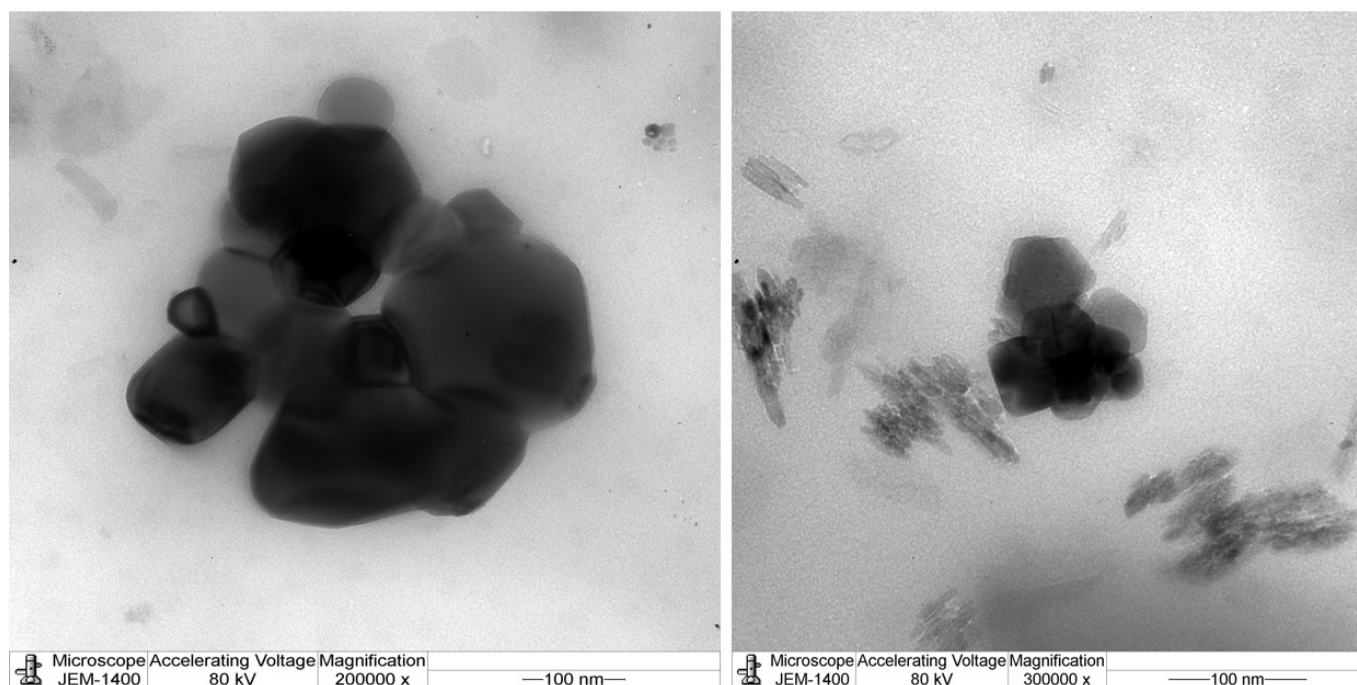


Figure 3. Transmission electron microscopy (TEM) images of freeze-dried mangosteen peel nanoparticles.

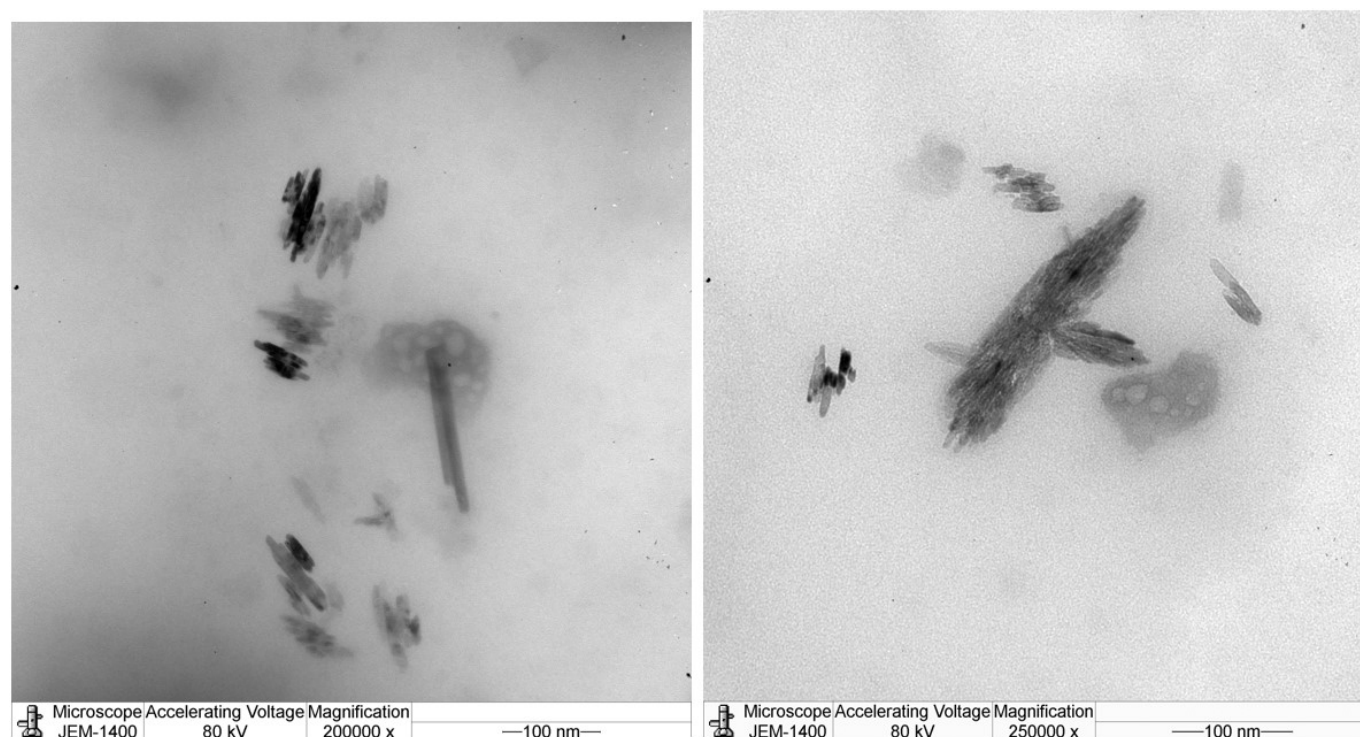


Figure 4. Transmission electron microscopy (TEM) images of air-dried mangosteen peel nanoparticles.

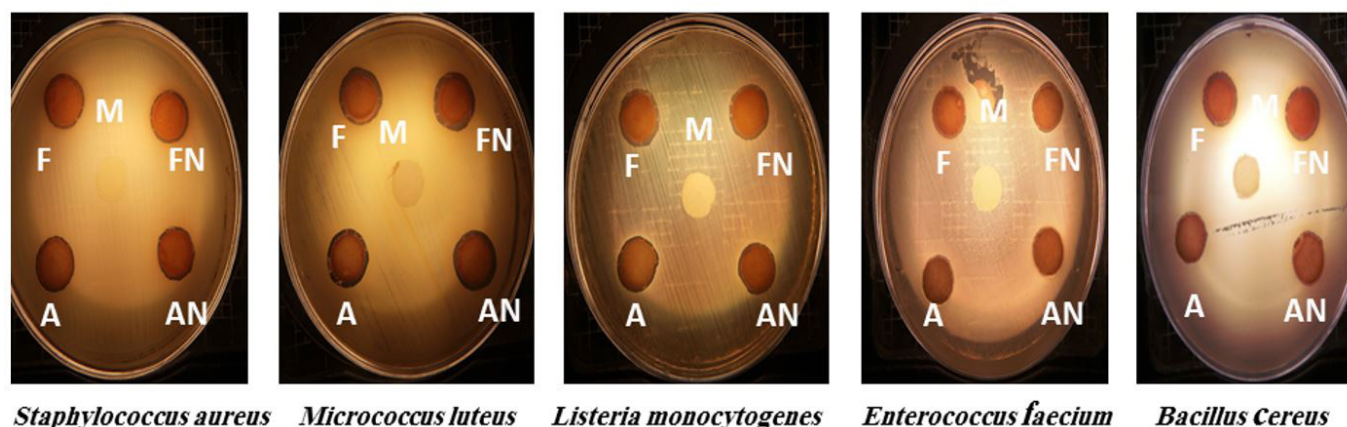


Figure 5. Zone of inhibition of discs saturated with freeze dried mangosteen nanoparticle (FN), normal (F), air dried mangosteen nanoparticle (AN), and normal (A) against various gram-positive bacteria. M indicates a disc saturated with methanol as a control.

Table 1. Content of total phenols, flavonoids, and antioxidant activity of flavonoids free-radical scavenging (DPPH and ABTS) and ferric reducing antioxidant power (FRAP) in methanol-extracted air- and freeze-dried mangosteen peel nanoparticles.

Name of sample	T. Phenols (mg gallic acid/g sample)	T. Flavonoids (mg catechin/g sample)	DPPH (%)	ABTS (g trolox/g sample)	FRAP (g trolox/g sample)
	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD
Air-dried mangosteen peel	401.303 $\pm$ 14.498	1.057 $\pm$ 0.119	94.775 $\pm$ 0.122	13.075 $\pm$ 0.534	5.728 $\pm$ 0.152
Air-dried mangosteen peel nanoparticles	479.744 $\pm$ 2.564	4.711 $\pm$ 0.207	94.303 $\pm$ 0.074	16.069 $\pm$ 0.424	6.696 $\pm$ 0.085
Freeze-dried Mangosteen peel	815.311 $\pm$ 3.935	15.394 $\pm$ 1.496	94.643 $\pm$ 0.128	29.943 $\pm$ 0.058	12.982 $\pm$ 0.368
Freeze-dried mangosteen peel nanoparticles	1112.646 $\pm$ 1.842	14.154 $\pm$ 0.119	95.707 $\pm$ 0.070	42.753 $\pm$ 0.200	17.806 $\pm$ 0.056

Values are means of three replicates. Results were given as mean (M) $\pm$ standard deviation (SD).

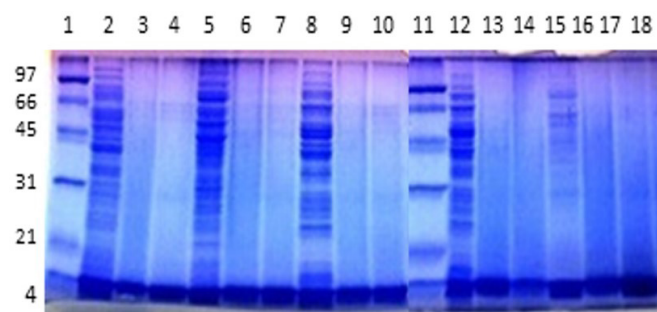


Figure 6. Total protein profile of various Gram-positive pathogenic bacteria treated with mangosteen peel nanoparticles (air- and freeze-dried) on 12% SDS-PAGE. Lane 1 and 11= protein marker, lane 2 = *Staphylococcus aureus* ATCC 29737 (Total protein). Lanes 3, 6, 9, 14, and 17 = air-dried mangosteen nanoparticles. Lanes 4, 7, 10, 15, and 18 = freeze-dried mangosteen nanoparticles. Lane 5 = *Bacillus cereus* ATCC 11778 (Total protein). Lane 8 = *Listeria monocytogenes* ATCC 19114 (Total protein). Lane 12 = *Enterococcus faecium* (Total protein). Lane 15 = *Micrococcus luteus* (Total protein).

Both freeze- and air-dried mangosteen peel nanoparticles (methanol-extracted) induced a large inhibition zone against all gram-positive bacteria tested.

The nanomangosteen peel extract showed antibacterial activity stronger impact to 3 tested bacterial: *S. aureus*, *B. cereus*, and *Shigella flexinera* than 40 mesh and 20 mesh size, however its encapsulated form has not shown satisfactory antibacterial activity. The PN has particle size of 308.30 nm, lower than AN and BN, respectively with polydispersity index (PI) of 0.14 (Sitti et al., 2018). Alkhuriji et al. (2020), investigated the oral administration of natural products biosynthesized silver nanoparticles (Ag-NPs) using *Garcinia mangostana* peel extract which could inhibit oral infection of *Listeria monocytogenes* in BALB/c mice.

SDS- PAGE

The results presented in Figure 6 illustrate the effect of mangosteen peel nanoparticles on the growth and protein formation of various Gram-positive bacteria as tested on SDS-polyacrylamide gel. The whole proteins of all bacterial strains tested almost completely vanished in all lanes of both the air- and freeze-dried mangosteen nanoparticles (3,4,6,7,9,10,13,14,16 and 17) in comparison with the control strain exhibiting all protein bands (lanes 2,5, 8, 12, and 15).

Interactions of free silver ions of silver nitrate with vital enzymes of bacteria provides antibacterial activity due to the phytochemicals particularly mangosteen the major compound which plays a vital role. Moreover the activity is happen due to the destruction of cell wall as well as DNA damage (Marambio-Jones & Hoek, 2010)

4 Conclusion

The use of novel technologies in the manufacture of mangosteen peel nanoparticles in combination with a freeze-drying process

may yield higher quality particles with better activity against Gram-positive bacteria than those obtained by using air-dried or normal extraction processes. These extracts may function as novel bactericides, as they inhibit the growth of bacteria on media as well as bacterial protein formation. The usage of mangosteen peels nanoparticles from the plant takes full advantage of unwanted waste material which is economically friendly, efficient, and safe. The synthesized mangosteen peel nanoparticles by the previous methods are potential to be applied in biomedical and other applications where nontoxicity is crucial.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study are included within the article.

Authors' Contributions

H.M.Y. supervised the experimental activity and wrote the manuscript with M.A.G who synthesized nanoparticles of mangosteen, M.F.S.E. determined the antioxidant activity, H.S.A. freeze-dried the mangosteen nanoparticles, R.A.A., M.F.E., M.A.A. and H.A. technical support for SEM analyses.

Acknowledgements

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R23), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

References

- Akillioglu, H. G., & Karakaya, S. (2010). Changes in total phenols, total flavonoids, and antioxidant activities of common beans and pinto beans after soaking, cooking, and in vitro digestion process. *Food Science and Biotechnology*, 19(3), 633-639. <http://dx.doi.org/10.1007/s10068-010-0089-8>.
- Alkhuriji, A. F., Majrashi, N. A., Alomar, S., El-Khadragy, M. F., Awad, M. A., Khatab, A. R., & Yehia, H. M. (2020). The beneficial effect of eco-friendly green nanoparticles using *Garcinia mangostana* peel extract against pathogenicity of *Listeria monocytogenes* in female BALB/c mice. *Animals (Basel)*, 10(4), 573. <http://dx.doi.org/10.3390/ani10040573>. PMID:32235366.
- Allahverdiyev, A. M., Abamor, E. S., Bagirova, M., Ustundag, C. B., Kaya, C., Kaya, F., & Rafailovich, M. (2011). Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light. *International Journal of Nanomedicine*, 6, 2705-2714. <http://dx.doi.org/10.2147/IJN.S23883>. PMID:22114501.
- Ayman, E. L., Hassan, S. M., & Osman, H. E. H. (2019). Mangosteen (*Garcinia mangostana* L.). In S. Nabavi & A.S. Silva (Eds.), *Nonvitamin and nonmineral nutritional supplements* (pp. 313-319). London, UK: Academic Press.
- Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*, 9(4), 449-454. <http://dx.doi.org/10.1016/j.jtusci.2014.11.001>.

- Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493-496. http://dx.doi.org/10.1093/ajcp/45.4_ts.493. PMID:5325707.
- Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. <http://dx.doi.org/10.1006/abio.1996.0292>. PMID:8660627.
- Chen, L. G., Yang, L. L., & Wang, C. C. (2008). Anti-inflammatory activity of mangostins from *Garcinia mangostana*. *Food and Chemical Toxicology*, 46(2), 688-693. <http://dx.doi.org/10.1016/j.fct.2007.09.096>. PMID:18029076.
- Dkhil, M. A., Moneim, A. E., & Al-Quraishy, S. (2016). Indigofera oblongifolia ameliorates lead acetate-induced testicular oxidative damage and apoptosis in a rat model. *Biological Trace Element Research*, 173(2), 354-361. <http://dx.doi.org/10.1007/s12011-016-0689-0>. PMID:27052307.
- El-Khadragy, M., Alolayan, E. M., Metwally, D. M., El-Din, M. F. S., Alobud, S. S., Alsultan, N. I., Alsaif, S. S., Awad, M. A., & Abdel Moneim, A. E. (2018). Clinical efficacy associated with enhanced antioxidant enzyme activities of silver nanoparticles biosynthesized using moringa oleifera leaf extract, against cutaneous leishmaniasis in a murine model of leishmania major. *International Journal of Environmental Research and Public Health*, 15(5), 1037. <http://dx.doi.org/10.3390/ijerph15051037>. PMID:29786651.
- Faried, M., Shameli, K., Miyake, M., Hajalilou, A., Kalantari, K., Zakaria, Z., Hara, H., & Khairudin, N. B. A. (2016). Synthesis of silver nanoparticles via green method using ultrasound irradiation in seaweed *Kappaphycus alvarezii* media. *Research on Chemical Intermediates*, 42(12), 7991-8004. <http://dx.doi.org/10.1007/s11164-016-2574-z>.
- Fu, C., Loo, A. E., Chia, F. P., & Huang, D. (2007). Oligomeric proanthocyanidins from mangosteen pericarps. *Journal of Agricultural and Food Chemistry*, 55(19), 7689-7694. <http://dx.doi.org/10.1021/jf071166n>. PMID:17715900.
- Gouveia, S., & Castilho, P. C. (2011). Antioxidant potential of *Artemisia argentea* L'Hér alcoholic extract and its relation with the phenolic composition. *Food Research International*, 44(6), 1620-1631. <http://dx.doi.org/10.1016/j.foodres.2011.04.040>.
- Gutierrez-Orozco, F., & Failla, M. L. (2013). Biological activities and bioavailability of mangosteen xanthones: a critical review of the current evidence. *Nutrients*, 5(8), 3163-3183. <http://dx.doi.org/10.3390/nu5083163>. PMID:23945675.
- Jung, H. A., Su, B. N., Keller, W. J., Mehta, R. G., & Kinghorn, A. D. (2006). Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen). *Journal of Agricultural and Food Chemistry*, 54(6), 2077-2082. <http://dx.doi.org/10.1021/jf052649z>. PMID:16536578.
- Lee, K. X., Shameli, K., Miyake, M., Kuwano, N., Khairudin, N. B., Bt A., Mohamad, S. E. Bt. & Yew, Y. P. (2016). Green synthesis of gold nanoparticles using aqueous extract of *Garcinia mangostana* fruit peels. *Journal of Nanomaterials*, 2016, 8489094. <http://dx.doi.org/10.1155/2016/8489094>.
- Marambio-Jones, C., & Hoek, E. M. V. (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research*, 12(5), 1531-1551. <http://dx.doi.org/10.1007/s11051-010-9900-y>.
- Nazre, M. (2014). New evidence on the origin of mangosteen (*Garcinia mangostana* L.) based on morphology and ITS sequence. *Genetic Resources and Crop Evolution*, 61(6), 1147-1158. <http://dx.doi.org/10.1007/s10722-014-0097-2>.
- Palapol, Y., Ketsa, S., Stevenson, D., Cooney, J. M., Allan, A. C., & Ferguson, I. B. (2009). Colour development and quality of mangosteen (*Garcinia mangostana* L.) fruit during ripening and after harvest. *Postharvest Biology and Technology*, 51(3), 349-353. <http://dx.doi.org/10.1016/j.postharvbio.2008.08.003>.
- Pothitirat, W., Chomnawang, M. T., Supabphol, R., & Gritsanapan, W. (2009). Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. *Fitoterapia*, 80(7), 442-447. <http://dx.doi.org/10.1016/j.fitote.2009.06.005>. PMID:19524646.
- Rajakannu, S., Shankar, S., Perumal, S., Subramanian, S., & Dhakshinamoorthy, G. P. (2015). Biosynthesis of silver nanoparticles using *Garcinia mangostana* fruit extract and their antibacterial, antioxidant activity. *International Journal of Current Microbiology and Applied Sciences*, 4, 944-952.
- Sitti, R. H. S., Sugita, P., Ambarsari, L., & Rahayu, D. U. C. (2018). Antibacterial Mangosteen (*Garcinia mangostana* Linn.) peels extract encapsulated in Chitosan. *Journal of Physics: Conference Series*, 1116, 042037. <http://dx.doi.org/10.1088/1742-6596/1116/4/042037>.
- Suksamrarn, S., Suwannapoch, N., Ratananukul, P., Aroonlerk, N., & Suksamrarn, A. (2002). Xanthones from the green fruit hulls of *Garcinia mangostana*. *Journal of Natural Products*, 65(5), 761-763. <http://dx.doi.org/10.1021/np010566g>. PMID:12027762.
- Tsai, S. Y., Chung, P. C., Owaga, E. E., Tsai, I. J., Wang, P. Y., Tsai, J. I., Yeh, T. S., & Hsieh, R. H. (2016). Alpha-mangostin from mangosteen (*Garcinia mangostana* Linn.) pericarp extract reduces high fat-diet induced hepatic steatosis in rats by regulating mitochondria function and apoptosis. *Nutrition & Metabolism*, 13, 88. <http://dx.doi.org/10.1186/s12986-016-0148-0>. PMID:27980597.
- Wu, C. H., Murthy, H. N., Hahn, E. J., & Paek, K. Y. (2007). Improved production of caffeic acid derivatives in suspension cultures of *Echinacea purpurea* by medium replenishment strategy. *Archives of Pharmacal Research*, 30(8), 945-949. <http://dx.doi.org/10.1007/BF02993961>. PMID:17879746.
- Yehia, H. M., & Al-Dagal, M. M. (2014). Prevalence of *Campylobacter jejuni* in chicken produced by major poultry companies in Saudi Arabia. *International Journal of Food Contamination*, 1, 2. <http://dx.doi.org/10.1186/s40550-014-0002-y>.
- Zadernowski, R., Czaplicki, S., & Naczek, M. (2009). Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). *Food Chemistry*, 112(3), 685-689. <http://dx.doi.org/10.1016/j.foodchem.2008.06.030>.
- Zarena, A. S., & Sankar, K. U. (2009). A study of antioxidant properties from *Garcinia mangostana* L. peel extract. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 8, 23-34.
- Zarena, A. S., & Udaya Sankar, K. (2012). 2011. Isolation and identification of pelargonidin 3-glucoside in mangosteen pericarp. *Food Chemistry*, 130(3), 665-670. <http://dx.doi.org/10.1016/j.foodchem.2011.07.106>.