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# Control some foodborne pathogens, contaminated bacteria and fungi by fabrication calyx cape gooseberry (*Physalis peruviana* L.) nanoparticles

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#### Abstract

We studied the effect of the calyx of cape gooseberry nanoparticles manufactured by a novel technique. There are several methods, such as jet mill, spray freezing, supercritical anti-solvent technique (SAS), and high-pressure homogenization, available for the production of drug micro/nanoparticles to improve drug solubility. Anti-solvent precipitation is also used to prepare micro- and nanosized particles to explain their effects on different foodborne pathogens, contaminated bacteria, and some fungi. Results indicated that nanoparticles of the cape gooseberry calyx contained higher antioxidant activity than that found in the normal calyx, seed, and juice. While studying the effect of cape gooseberry calyx nanoparticles on microorganisms, it was found that the zone of inhibition was highly positive against *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Salmonella typhimurium* compared to that of the normal cape gooseberry calyx. All species of molds, including *Mucor sp.*, *Aspergillus niger*, *Penicillium sp.*, and *Alternaria sp.*, and yeasts like *Saccharomyces cerevisiae* and *Rhodotorula glutinis* were affected by nanoparticles of the cape gooseberry calyx compared to those of the normal calyx.

Keywords: calyx; cape gooseberry; nanoparticles; antioxidant activities; microorganisms.

**Practical Application:** Calyx Cape Gooseberry (*Physalis peruviana* L.) Nanoparticles can be used as antangonistic effect against foodborne pathogenic bacteria.

#### **1** Introduction

The cape gooseberry (Physalis peruviana L.) is an annual plant belonging to the family Solanaceae, and it is a short-lived perennial that can grow up to one meter (Ramadan, 2011). It contains vitamins A and C, some of the vitamin B-complexes, and minerals, such as phosphorus, iron, potassium, and zinc Salazar et al., 2008). The nutritional composition and bioactive components found in gooseberries confirm its main benefits (Hassanien, 2001). Fruit juices are complex and heterogeneous colloidal insoluble particles dispersed in a continuous medium rich in soluble compounds, including sugars, organic acids, soluble pectins, phenolic compounds, and salts (Eraso-Grisales et al., 2021). Antioxidant contents and high levels of vitamin E of gooseberry have considerable functional properties (Ramadan & Moersel, 2009). Phenolic compounds of gooseberry have been extensively studied for their health properties from the epidemiological point of view, and their functionality depends on their content, intake, and bioavailability. Bioavailability of these compounds can also be affected by microstructure and binding of phenolic compounds within the food matrix, which are directly related to fruit processing (Balasundram et al., 2006).

The calyx of cape gooseberry contains five hairy sepals and veins and has a length of about 4–5 cm after fecundation that

completely cover the fruit during development and growth (Flórez et al., 2000). At the beginning through 40–45 d of development, it appears green. However, after fruit ripening, it loses chlorophyll and turns yellow-orange. At this stage, it can protect the fruits from birds, insects, extreme climate conditions (including cold, heat, and hail), and mechanical damage as well as serve as an indispensable source of carbohydrates for the first 20 d of fruit growth (Fischer et al., 2000).

In the inner base of the calyx, a glandular tissue that produced a terpenic resin when the fruit had a diameter of 10–11 mm, which covers the fruit up to its mature stage (Baumann & Meier, 1993). This researcher believed that the resin could be a repellent against insects. Further, some substances, such as withanolide E and 4 B-hydroxywithanolide, have been found to increase surrounding the calyx in contact with the fruit, and during maturation, at a concentration that leads to an anti-feeding effect (Franco et al., 2007). Currently, the market requires the commercialization of fruit without calyx, which reduces the shelf life of the fruits (Cárdenas-Barboza et al., 2021). Although Olivares-Tenorio et al. (2017) mentioned that under storage conditions at 12 °C cape gooseberry with calyx has a shelf life of 24 days, and fruits without calyx only retain

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their quality postharvest for 11 days. So there are necessary to manufacture of calyx nanoparticle for using these leaves in the production of components has benefits to human.

Nanoparticle compounds research is a new heavily investigated area due to their potential in improving the properties of materials. Combination between high-antioxidant materials and nanoparticles is a new branch of inquiry to help scientists control the growth and distribution of microorganisms.

Therefore, the aim of this study was to manufacture nanoparticles of cape gooseberry calyx with dimensions of less than 100 nm using the new nanonization (reduction in size) method to control or limit the growth of known microorganisms distributed in nature and to study their antioxidant activity.

### 2 Materials and methods

#### 2.1 Sample preparation

The fruits of *P. peruviana* L. were purchased from a local market in Riyadh city, Saudi Arabia. The calyx was separated from the fruits, cut into small pieces, and dried in a dry oven at 60 °C. The dried leaves were soaked in methanol (100 mg/mL) and left overnight in a refrigerator. The mixture was then centrifuged at 5000 rpm for 5 min. The supernatant was used directly and tested against microorganisms. The fruits were blended, and the seeds and juice were separated using a small sieve.

#### 2.2 Fabrication of calyx cape gooseberry nanoparticles

Calyx cape gooseberry powder (300 mg) was added to 20 mL of methanol while stirring. Following, under ultrasonic conditions, this solution was sprayed into boiling water (40 mL) in a dropwise manner at a flow rate of 0.2 mL/min in 5 min using an ultrasonic power of 750 W and a frequency of 20 kHz. After sonication for 10 min, the contents were stirred at 200–800 rpm at room temperature (20 °C) for approximately 20 min. The solution was dried to obtain Calyx Cape Gooseberry as nanoparticles.

# 2.3 Antioxidant activities

#### Total phenolic compounds

Total content of phenolic compounds in methanolic extracts was determined by the Folin–Ciocalteu method as described by Wu et al., 2007, briefly, 2.5 mL of distilled water and 0.1 mL of sample extract were added to a test tube, followed by addition of 0.1 mL of undiluted commercially available Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA). The solution was mixed well and allowed to stand for 6 min before 0.5 mL of a 20% sodium carbonate solution was added. The color was allowed to develop for 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 of gallic acid solutions and expressed as gallic acid equivalents per gram of dry weight sample.

#### Total flavonoids content

The total flavonoid content of crude extract was determined by the aluminum chloride colorimetric method according to Baba & Malik (2015), In brief, 50  $\mu$ L of crude extract was mixed with 4 mL of distilled water followed by 0.3 mL of 5% NaNO<sub>2</sub> solution. Further, 0.3 mL of 10% AlCl<sub>3</sub> solution was added after 5-min incubation, and the mixture was allowed to stand for 6 min. Subsequently, 2 mL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 10 mL with distilled water. The mixture was allowed to stand for 15 min, and the 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 and Karakaya, 2010. A 0.08 mM DPPH radical solution in methanol was prepared. Following, 950 µL of DPPH stock solution was added to 50 µL extract and incubated for 5 min. At precisely 5 min later, absorbance readings of the mixture were performed at 515 nm (Cary 50 Scan; Varian). Antioxidant activity (AA) was expressed as percentage inhibition of DPPH radical using the following equation:  $AA = 100 - [100 \times (A_{sample}/A_{control})]$ , 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<sup>++</sup> assay was performed according to the method of Gouveia and Castilho, 2011. The ABTS<sup>++</sup> radical solution was prepared by reacting 50 mL of 2 mM ABTS solution with 200  $\mu$ L of 70 mM potassium persulfate solution. This mixture was stored in the dark for 16 h at room temperature, and it remained stable in this form for two days. For each analysis, the ABTS<sup>++</sup> 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 freshly prepared for each set of analyses. To determine the antiradical scavenging activity, an aliquot of 100  $\mu$ L methanolic solution was added to 1.8 mL of ABTS<sup>++</sup> solution, and the absorbance decrease, at 734 nm, was recorded during 6 min. Results were expressed as  $\mu$ mol Trolox equivalent per g of dried sample (mmol eq. Trolox/g), based on the Trolox calibration curve.

#### Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) was performed according to the procedure described by Benzie & Strain, 1996. The FRAP reagent included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl<sub>3</sub> at the ratio 10:1:1 (v/v/v). Briefly, 3 mL of the FRAP reagent was mixed with 100  $\mu$ L of the sample extract in a test tube and vortexed in a 37 °C incubator for 30 min in a water bath. Reduction of ferric-tripyridyltriazine to the ferrous complex formed an intense blue

color, which was measured using a UV-vis spectrophotometer (Varian Cary 50) at 593 nm at the end of 4 min. Results were expressed in terms of mmol Trolox equivalent per g of dried sample (mmol eq. Trolox/g).

#### 2.4 Antimicrobial assays

#### Test microorganisms

The following microorganisms were tested: *Bacillus cereus* ATCC 14579, *Salmonella typhimurium* ATCC 14028, *Listeria monocytogenes* ATCC 19114, *Bacillus subtilis* (local isolate), *Staphylococcus aureus* ATCC 29737, *Clostridium perfringens* ATCC 13124, *Escherichia coli* ATCC 10537, *Micrococcus luteus* (local isolate), *Saccharomyces cerevisiae* DSMZ 1333, *Rhodotorula glutinis* (local isolate), *Mucor sp.* (local isolate), *Aspergillus niger* (local isolate), *Penicillium sp.* (local isolate), and *Alternaria sp.* (local isolate).

#### Agar well diffusion method

Agar diffusion method was used to determine the antimicrobial activity of Calyx Cape Gooseberry nanoparticles against some foodborne pathogens, contaminated bacteria, and fungi, which are listed above. Briefly,  $100 \mu L-10^6/mL$  of each active bacterial strain, which were grown on Brain Heart Infusion agar (Oxoid CM 1136) for 24 h at 37 °C, were spread on the surface of Muller

Hinton agar plates (Oxoid CM 0337). Further,  $50 \mu g/mL$  of cape gooseberry calyx nanoparticles and normal calyx were dissolved in methanol and left overnight in a refrigerator. Subsequently, three holes using a 6-mm diameter sterile cork borer were punched on the agar. One hole contained a volume of  $100 \mu L$  of cape gooseberry calyx nanoparticles, second contained  $100 \mu L$  of normal cape gooseberry calyx, and the third contained only  $100 \mu L$  methanol as a control. Finally, the agar plates were incubated at 37 °C for 24 h, and the effects of calyx cape gooseberry nanoparticles and normal calyx (measured as zone of inhibition) were recorded as positive and negative results.

#### 3 Results and discussion

Figure 1 shows the morphology of the resulting nanoparticles as investigated by transmission electron microscopy (TEM). TEM images revealed that a thin layer of organic material surrounded the nanoparticles, which is indicative of the cape gooseberry calyx. Furthermore, the resulting calyx cape gooseberry nanoparticles had irregular spherical shapes due to aggregation.

The size of the synthesized nanoparticles was analyzed through Zetasizer (Figure 2). The average particle sizes (diameter) varied from about 1 nm to 261 nm, and the Polydispersity Index (PDI) was 0.3, which represents mono-dispersity as observed clearly from the peak indicating particle stability.



# A

В

**Figure 1**. Transmission electron microscopy (TEM). Microphotograph of calyx cape gooseberry synthesized nanoparticles at  $10000 \times (A)$  and  $250000 \times (B)$  magnifications.

Data in Table 1 show the total phenols in normal calvx of cape gooseberry at  $42.385 \pm 0.402$  mg gallic acid/g sample. This quantity was higher than found in both bulbs and seeds, which were  $4.547 \pm 0.080$  and  $3.350 \pm 0.160$ , respectively. Further, total flavonoids of normal calyx of cape gooseberry, determined as mg catachin and rutin/g sample, were 2.058  $\pm$  0.149 and  $19.196 \pm 1.648$ , respectively, but were not detected in both bulbs and seeds. Similar antioxidant activity of flavonoids freeradical scavenging agents (DPPH and ABTS) of normal calyx of cape gooseberry was  $32.75 \pm 0.289\%$  and  $0.8601 \pm 0.0095$  g (g trolox/g sample). In contrast, DPPH found in bulbs and seeds was  $21.72 \pm 0.328$  and  $16.48 \pm 0.289\%$ , and ABTS was  $0.2105 \pm 0.0022$  and  $0.1521 \pm 0.0015$  g (Trolox/g sample), respectively. Ferric reducing antioxidant power (FRAP) found in normal calyx of cape gooseberry was  $0.5373 \pm 0.0126$  g (Trolox/g sample). For bulbs and seeds, values were 0.0764  $\pm$  0.0037 and  $0.0498 \pm 0.0020$  g (Trolox/g sample), respectively. From the above data, it is evident that normal calyx of cape gooseberry contained higher antioxidant capacity than found in bulbs and seeds. As a result, we selected the calvx to prepare nanoparticles, and the results showed that its antioxidant capacity increased 2-fold compared to normal calyx for total phenols, DPPH, ABTS, and FRAB, which were  $85.088 \pm 4.093$  (mg Gallic acid/g sample),  $69.517 \pm 1.131\%$ ,  $1.667 \pm 0.012$  (g Trolox/g sample), and  $1.604 \pm 0.180$  (g Trolox/g sample), respectively. For verification that the effect of calyx nanoparticles not due to antioxidants alone after nantechnique method, the nanoparticles frees filtration was



**Figure 2**. Zetasizer analysis. Measurement of formed Calyx cape gooseberry nanoparticles size by zetasizer.

tested against microorganisms. The same inhibition zone was noticed against microorganisms. The calyx's cape gooseberry, a material that encloses the fruit, protecting it from insects, birds, diseases, and adverse weather conditions, is considered as a post-harvest waste. However, infusions of this material are used in traditional medicine to reduce the symptoms of arthritis (Wei et al., 2012; Simbaqueba et al., 2011; Wu et al., 2005; Yen et al., 2010). In previous studies, an important antioxidant and anti-inflammatory activity was obtained from leaves and stems of this species, associated with the presence of flavonoids and other compounds with antioxidant properties (Wu et al., 2005; Fischer et al., 2000; Hassanien & Moersel, 2003; Wu et al., 2006). Some authors studies the effect of extracts of cape gooseberry and appeared a protective effect in liver and inhibit cancer cell lines growing and showed anti-inflammatory activity (Puente et al., 2011; Al-Olayan et al., 2014; Çakir et al., 2014).

Therefore, nanoparticles of the calyx of cape gooseberry for activity against some foodborne pathogens, contaminated bacteria, molds, and yeasts, as shown in Table 2 found that almost all bacterial strains, molds, and yeasts were affected by calyx nanoparticle compared to the normal calyx due to its high antioxidant activity.

Many studies reported the contents of cape gooseberry and its biological activities in extracts of fruits, stems, leaves, and calyx. Folk medicine of most organs of cape gooseberry, including leaves, stems, and roots, have been widely used because of their diuretic, antiseptic, antifungal, antibacterial, anti-carcinogenic, antimalarial and anti-inflammatory effects (Puente et al., 2011; Wu et al., 2007; Akillioglu & Karakaya, 2010) Some authors have studied the effect of extracts of cape gooseberry and have shown a protective effect in the liver, growth inhibition in cancer cell lines, and anti-inflammatory activity (Gouveia & Castilho, 2011; Benzie & Strain, 1996).

The anti-inflammatory activity of leaf and plant stem extracts from *Physalis peruviana* has been confirmed mainly in macrophages (Benzie & Strain, 1996). Another study of *Physalis peruviana* ethanol extracts from leaves and shoots—external material is not edible and provides separate protection of the fruit also known as the calyx—showed antibacterial activity, antioxidant activity, and protection against DNA damage by controlling the expression of Bcl-2 family genes, which regulate apoptosis in HeLa (Tapia & Fries, 2007). Regarding the study

Table 1. Content of total phenols, flavonoids and antioxidant activity of flavonoids free-radical scavenging (DPPH and ABTS) and ferric reducing antioxidant power (FRAP) found in the bulb, seed and calyx of cape gooseberry (*Physalis peruviana*).

				_		
Cape gooseberry	T. Phenols (mg	T. Flavonoids	T. Flavonoids	DPPH (%)	ABTS (g	FRAB (g
	Gallic acid /g	(mg Catachin /g	(mg Rutin/g		Trolox/g sample)	Trolox/g sample)
	sample)	sample)	sample)		5 1 /	0 1 /
	$M \pm SD$	$M \pm SD$	$M \pm SD$	$M \pm SD$	$M \pm SD$	$M \pm SD$
Normal						
Cape gooseberry calyx	$42.385 \pm 0.402$	$2.058 \pm 0.149$	$19.196 \pm 1.648$	$32.75\pm0.289$	$0.8601 \pm 0.0095$	$0.5373 \pm 0.0126$
Cape gooseberry bulbs	$4.547\pm0.080$	ND	ND	$21.72\pm0.328$	$0.2105 \pm 0.0022$	$0.0764 \pm 0.0037$
Cape gooseberry seeds	$3.350\pm0.160$	ND	ND	$16.48\pm0.289$	$0.1521 \pm 0.0015$	$0.0498 \pm 0.0020$
Nanoparticle						
Cape gooseberry calyx	$85.088 \pm 4.093$	$0.377\pm0.024$	$0.609 \pm 0.264$	$69.517 \pm 1.131$	$1.667\pm0.012$	$1.604\pm0.180$

		Methanolic extraction		
Extractions Microorganisms	*G+/G-/Y/M	Normal calyx of cape gooseberry (100 μL)	Nanoparticle calyx of cape gooseberry (100 μL )	
B. cereus ATCC 14579	G+	+	+++	
S. typhimurium ATCC 14028	G-	+	++	
L. monocytogenes ATCC 19115	G+	-	++	
B. subtilis (local isolate)	G+	+	+++	
Clostridium perfringens ATCC13124	G+	+	+++	
<i>E. coli</i> ATCC 10537	G-	-	++	
Staph. aureus ATCC 29737	G+	+	+++	
Mucor sp. (local isolate)	М	+	+++	
Aspergillus niger (local isolate)	М	+	+++	
Penicillium sp.(local isolate)	М	+	+++	
Alternaria sp.(local isolate)	М	+	+++	
Rhodotorula glutinis (local isolate)	Y	+	++	
Saccharomyces cerevisiae DSMZ 1333	Y	+	+++	
Micrococcus luteus (local isolate)	G+	+	+++	

Table 2. Comparison between effect of methanolic extract of calyx of cape gooseberry nanoparticles and normal calyx on Gram positive, negative bacterial strains, molds, and yeasts.

\*G+: Gram positive bacteria; G-: Gram negative bacteria; Y: Yeast; M: Mold.

of the calyces of cape gooseberry, there are a few reports on the effect of normal calyx; however, no studies are related to the effect of nanoparticles on microorganisms.

# **4** Conclusions

Regarding the study of the calyces of cape gooseberry, few studies have been conducted on the normal calyx and none related to nanoparticles. We can conclude that the methanolic extract of the calyx of cape gooseberry nanoparticles demonstrated good action potential against some Gram-positive and negative bacteria, most molds, and yeasts compared to the normal calyx. The effect of calyx cape gooseberry nanoparticles is due to its high antioxidant capacity, suggesting that it could play an important role in antimicrobial activity and be applied in different fields.

# **Conflict of interest**

The authors declare no conflict of interest.

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