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In vitro digestibility of *Aucklandia costus*-loaded nanophytosomes and their use in yoghurt as a food model

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ABSTRACT

This work achieved the encapsulation of valuable bioactive substances from *Aucklandia costus* (AC) in nanophytosomes as a new phytoconstituent delivery system. Supercritical fluid extraction (SFE) and solvent-maceration methods were used to extract oil and phenolics from AC, respectively. The physicochemical characterization of SFE-oil and phenolic extract (PE)- High-performance liquid chromatography (HPLC) and Gas chromatography–mass spectrometry (GC-MS) were used to identify and quantify all compounds extracted from AC using both extraction methods. loaded nanophytosomes was studied using dynamic light scattering (DLS), encapsulation efficiency (EE), and transmission electron microscopy (TEM). The *in vitro* digestibility of SFE-oil and PE-loaded nanophytosomes after *in vitro* digestion was determined. Yoghurt was selected as a food model for fortification with AC-loaded nanophytosomes at different concentrations (5%, 10%, and 15%). pH, acidity, water holding capacity (WHC), and viscosity were evaluated in all yoghurt samples during storage, while color analysis and sensory evaluation were evaluated at 0 days. Optimized SFE-oil- and PE-loaded nanophytosomes showed promising results, and the bioavailability suggested that nanoencapsulation provided a controlled release of the phenolic and flavonoid compounds. Moreover, the addition of AC-loaded nanophytosomes into yoghurt resulted in increases in the viscosity (16.53 Pa s^{-1}) and decreases in WHC (59.50%). Yoghurts with AC-loaded nanophytosomes were more stable than the control (yoghurt without nanophytosomes). Overall, the current findings indicated that AC-loaded nanophytosomes would be a preferable candidate for incorporation into yoghurt to improve its quality. This research might provide scientific evidence supporting AC-loaded nanophytosomes as possible fortifying elements in manufacturing functional yoghurt and a new vision for designing unique dairy products with functional qualities.

1. Introduction

In recent decades, a strong partnership has developed between the World Health Organization (WHO), the Food and Drug Administration (FDA), and sources of medicinal firms across the globe to improve the usage of botanicals in Ayurveda medicine (Fridlender et al., 2015; Zaky et al., 2021). *Aucklandia costus* Falc. Syn. *Saussureacostus, Aplotaxislappa, and Saussurealappa* have a long history of 2500 years. Since Islamic culture, it has been extensively recognized as "Al-Kost Al-Hindi" in Arab countries and utilized as a traditional remedy (Abdallah et al., 2017; Jo et al., 2020). The root of *Aucklandia costus* was taken orally as a tea in Unaniayurvedic medicine to treat ulcers, gastritis, liver disorders, and diarrhea. However, root oil was used topically to treat herpes scabies and dermatitis (Pandey et al., 2007). Sesquiterpenes, triterpenes, and phenolics were found in the genus Costus. Sesquiterpenes are the most distinctive secondary plant metabolites of *Aucklandia costus* in terms of structure (Zhao et al., 2017). The primary compounds of the Indian species of A. lappa Decne were dehydrocostus lactone, caryophyllene oxide, and alloaromadendrene (Benedetto et al., 2019).

Because phytochemicals are very susceptible to high temperature and pH environments, the encapsulation technique may be employed to boost their constancy and reduce the disagreeable odor (Soliman et al., 2022; Stojanovic et al., 2012). Nanoencapsulation may increase the solubility and stability of bioactive substances in foods and drinks

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during storage (Durán & Marcato, 2013). It can also help to conceal off-flavor and strong scents, which can negatively influence the sensory qualities of goods (Saifullah et al., 2019; Zuidam & Heinrich, 2010). Phytosomes are a new lipid-based vesicle delivery method utilized in developing plant-based nutraceuticals and medications (Babazadeh et al., 2018; Ghanbarzadeh et al., 2016). The use of phytosomes in the encapsulation of bioactive substances from plant extracts increases their bioaccessibility, improves absorption in the digestive system, increases the antioxidant and antimicrobial activities of bioactive substances, and can protect nutraceuticals during food processing, such as pasteurization, sterilization (pH, heat treatment), and storage (Pawar & Bhangale, 2015). The improved stability of phytosomes is generally due to the formation of stable hydrogen bonds between phosphatidylcholine molecules and nutraceuticals, resulting in the formation of an aqueous and lipid-soluble complex (Ghanbarzadeh et al., 2016). Phytosomes have been recently used in clinical and pharmaceutical studies for phytoconstituents, such as silymarin (Maryana et al., 2016), curcumin (Zhang et al., 2013), sinigrin (Mazumder et al., 2016), rutin (Vankudri et al., 2016), and quercetin (Maramaldi et al., 2016).

However, phytosomes have received little attention as a new delivery vehicle in food products. The primary drawbacks of *Aucklandia costus* include its poor aroma, low-solubility aqueous phase, susceptibility to high temperature and alkaline conditions, and destruction during food manufacturing and storage, proving the need for innovative approaches, such as phytosome encapsulation (Kumar et al., 2020; Yang et al., 2020). The extraction procedure significantly impacts plants' phytochemical contents (Younis et al., 2021; Zaky et al., 2022). Accordingly, this study aimed to extract all constituents in *Aucklandia costus* utilizing supercritical fluid extraction and maceration and loaded them in a nanophytosome system. Since yoghurt is a staple food in several cultures, AC-loaded nanophytosomes were utilized as a food model to solve the primary issues.

2. Materials and methods

2.1. Materials

Dry roots of *Aucklandia costus* (AC) were purchased from a local market of medicinal plants in Cairo, Egypt. The Animal Production Research Institute, Agriculture Research Center, Dokki, Egypt, supplied low-fat buffalo milk. The traditional yoghurt starter culture consisted of *Streptococcus thermophilus* and *Lactobacillus delburkii* subsp. *bulgaricus* was obtained from Chr. Hansen laboratories (Chr. Hansen Holding A/S, 2970 Hoersholm, Denmark) and enumerated in UHT skimmed milk before use. Soy lecithin granules were obtained from Solgar, Inc. (Leonia, NJ 07605, USA). Merck Chemicals provided all chemicals (64293 Darmstadt, Germany).

2.2. Methods

2.2.1. Extraction of Aucklandia costus oil

According to Lammari et al. (2021), the dried powder (1 kg) of *Aucklandia costus* was subjected to supercritical fluid extraction (SFE) using a dynamic pilot plant (Separex 4343, type SF2) supplied by Separex 7 (Champigneulles, France). The operating extraction parameters were as follows: dynamic extraction time: 180 min; CO_2 flow rate: 50 g/min; CO_2 pressure: 100 bar; extraction temperature: 40 °C; and separation temperature: 30 °C.

2.2.2. Extraction of Aucklandia costus phenolics

Ten grams of *Aucklandia costus* were added to 200 mL of ethanol (70%), placed in an ultrasonicator for 1 min at room temperature (25 °C) and then centrifuged, and the supernatant was separated. The extraction procedure was conducted three times. The solvent was evaporated by a rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland), and the residue was powdered in a freeze drier (Labconco cooperation, Kansas City, USA) at –52 °C for 48 h under 0.1 mPa and stored at –18 °C until analysis (El-Messery et al., 2021a).

2.2.3. HPLC analysis

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using an Eclipse C18 column (4.6 mm × 250 mm i.d., 5 μm). An Agilent Zorbox SB-C18 column (250 × 4.6 mm i.d., 5 μm) was used at a column temperature of 30 °C. The filtered sample (20 μL) was injected, and the mobile phase consisted of 0.1% formic acid (A) and methanol (B) with a flow rate of 1.0 mL/min. The gradient was set as follows: 0 min 25% B; 20 min 25% B; 30 min 35% B; 40 min 100% B; 42 min 100% B; and 50 min 25% B. The peaks of the chromatogram were detected at 280 nm. The levels of each compound were quantified based on a standard curve, and the values were displayed as μg/g DW of *Aucklandia costus* (Zaky et al., 2020).

2.2.4. GC–MS analysis

The GC–MS system (Agilent Technologies, Santa Clara, California, USA) was equipped with a gas chromatograph (7890B) coupled with a mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt.

2.2.5. Preparation of nanophytosomes

SFE-oil and PE were encapsulated into nanophytosomes through the thin layer hydration technique using an equivalent weight ratio of SFE-oil or PE to soy lecithin (1:1, 1:2, and 1:3) (Babazadeh et al., 2017). Sixty milligrams of SFE-oil, PE and soy lecithin were dissolved in ethanol and placed in a round bottom flask on a vacuum rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland) for 60 min/60 °C to separate the ethanol and form a thin, dry film at the bottom of the round bottom flask. Then, the thin layer was hydrated in a rotary evaporator at 40 °C with 10 mL of distilled water. Probe sonication (160 W power, 20 k*Hz* frequency and with 50% pulse; Sonics and materials, Inc., Vibra, Cell, USA) was used to minimize the size of the initial phytosomes (10 min cycles with a 1-min rest between cycles).

2.2.6. Characterization of nanophytosomes

The surface charge (ζ-potential), particle diameter (*Z*-average) and polydispersity index (PDI) of the SFE extract and residual nanophytosomes were evaluated using the dynamic light scattering (DLS) method (Zetasizer Nano ZS, Malvern Panalytical Ltd, Malvern WR14, UK). Samples were prepared via dilution of an adequate volume of emulsion sample of approximately 0.1 mL, which was added to approximately 3.5 mL of distilled water. Then, the mixture was subjected to gentle sonication. The diluted sample was transferred to a 3 mL disposable PVC transparent cuvette and a 1 mL two electrode PVC disposal cell for zeta potential measurements. For each sample, a detection angle of 173° was chosen for the size measurement unless stated otherwise. The refractive index of the sample was approximately 1.47, and the optical absorption was adjusted to an absorbance of 0.1. To study and visualize the morphology of the nanophytosomes, TEM was used (JEM-2100 Electron Microscope, JEOL CO., Ltd., Beijing, China) after dry sample coating with gold (DST3, Nanostructured Co., Tehran, Iran).

2.2.6.1. Determination of total phenolic and flavonoid contents (TPC and TFC). The total phenolic content (TPC) of the samples was measured using the Folin-Ciocalteu reagent, according to Zaky et al. (2019). The calibration curve was utilized to quantify TPC, represented as mg of gallic acid equivalent per g of sample.

The total flavonoid content (TFC) was assayed using the aluminum chloride colorimetric technique of Chang et al. (2002). The rutin solution calibration standard was used to quantify the flavonoid concentration per g of sample, which was reported as mg of rutin equivalent (RE).

2.2.6.2. Encapsulation efficiency (EE). According to González-Ortega et al. (2021), the phytosomes were centrifuged (12,000×*g*, 180 min, 20 °C), the supernatant was separated and analyzed, and the pelleted phytosomes were resuspended in fresh distilled water. These resuspended phytosomes were disrupted by adding 1 mL methanol and 1 mL chloroform to determine the amounts of encapsulated compounds. The mixture was vortexed thoroughly and left to allow phase separation. The concentrations of phenolics in the upper water–methanol phase and in the supernatant were determined and considered as the encapsulated and nonencapsulated fractions, respectively. The encapsulation efficiency was calculated using Eq. (1):

$$\mathbf{EE}\ (\%) = \frac{\textbf{Mass of encapsulated phenolics}}{\textbf{Total mass of phenolics (encapsulated + nonencapsulated)}} \times \mathbf{100}$$

2.2.6.3. In vitro digestibility study. According to El-Messery et al. (2021b), SFE-oil and PE-loaded nanophytosomes were subjected to an *in vitro* digestion procedure simulating oral, gastric, and intestinal digestion. At 37 °C, all samples were digested with simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF). These fluids included calcium chloride and the appropriate enzyme(s). Initially, 1 g of each sample was orally digested with 5 mL of SSF containing 7.5 mg of amylase and 1.5 mM $CaCl_2$. This solution was mixed and incubated at 37 °C for 2 min after adjusting the pH to 7.5. The oral digestate was combined with 5 mL of SGF to begin the next step of gastric digestion (20 mg of pepsin and 0.15 mM of $CaCl_2$). The pH of the previous solution was modified to 2 and incubated for 2 h at 37 °C and 50 rpm in a shaking water bath. Then, the gastric digestate from the previous stage was combined with 10 mL SIF containing 0.6 mM $CaCl_2$, 37.5 mg pancreatin, and 40 mg bile salts. The pH of this combination was then changed to 7.5, and incubated under the same conditions. At the end of each digestion step, part of the soluble fractions was centrifuged at 15,000×*g* for 30 min at 25 °C, filtered and then collected to determine TPC and TFC.

2.2.7. Processing of stirred yoghurt

Fresh, low-fat buffalo milk with 0.5% fat, 3.5% protein, and 4.7% lactose was used to formulate yoghurt samples. The low-fat buffalo milk was pasteurized for 10 min at 95 °C before being cooled to 42 °C. The starter culture (S. thermophilus and lb. delburkii subsp. bulgaricus) was injected at a rate of 3% (*w/v*) and incubated at 42 °C for 3–4 h until the pH decreased to ~ 4.6 (Haji Ghafarloo et al., 2020). Based on the results of the highest encapsulation efficiency, F1 (PE and soy lecithin was 1:1) and F4 (SFE-oil and soy lecithin was 1:1) were selected, and a mixture formula (MF) was made in a ratio of 1:1 to maximize the benefits of all the active ingredients in both PE and SFE-oil. Yoghurt was fortified with three concentrations of MF-loaded nanophytosomes (5%, 10% and 15% w/w) and then stirred. The physical characteristics and sensory attributes were investigated for 15 days at 5 ± 2 °C.

2.2.7.1. pH and acidity. The pH of yoghurts was measured utilizing a pHmeter (microprocessor pH 211, HANNA Instruments Inc., Winsocket, USA) directly after manufacturing at 25 °C, while the acidity was determined using the titration technique (AOAC, 2000).

2.2.7.2. Apparent viscosity. The viscosity of yoghurt samples was measured using a Brookfield viscometer (Brookfield Model-LV; Brookfield Engineering Laboratory, Stoughton, USA). Shear rates ranging between 3 and 100 S^{-1} were applied to the samples.

2.2.7.3. Color. The color of yoghurt samples was analyzed using Hunter LAB (Color quest XE, Stotto Hunter Lab, Leicester LE4 3 EH, UK). The light source was Illuminant D65, and the viewing angle was 10°. L* (lightness), a* (negative value indicates green, positive value implies red), and b* (negative value means blue and positive value means yellow) were used to assess the color value.

2.2.7.4. Water holding capacity (WHC). The WHC was calculated using the Arslan and Ozel et al. (2012) method. Twenty-five grams of yoghurt sample (YS) were centrifuged for 10 min at 5000×*g*. The separated whey (SW) was quantified in g, and the WHC was determined as follows (Eq. 2):

$$\mathrm{WHC}(\%) = \frac{\mathrm{YS} - \mathrm{SW}}{\mathrm{YS}} \times 100$$

2.2.7.5. Sensory evaluation. A 10-point hedonic scale was employed for sensory assessment, including appearance, flavor and texture. Every yoghurt sample was evaluated separately, and the results were handed to the panelists in individual plastic cups. At each session, three-digit-coded yoghurts were randomly distributed to the panel group.

2.2.8. Statistical analyses

All yoghurt treatments were repeated three times, and the findings were calculated as the average standard deviation (SD). IBM SPSS Statistics (Version 25.0, IBM Corp., Armonk, NY, USA) was used to analyze the data, and Duncan's test was used at a 5% significance level.

3. Results and discussion

3.1. HPLC of phenolic extract (PE)

The PE of *Aucklandia costus* was identified and calculated by HPLC, as shown in Table 1 and Fig. S1. The obtained data indicated that the highest phenolic compounds were found in the order naringenin, caffeic acid, gallic acid, chlorogenic acid, rutin, and pyro catechol, followed by other compounds at minor concentrations. Deabes et al. (2021) reported that the phenolic compounds found in ethanol and ethyl acetate extracts of *Aucklandia costus* are naringenin, chlorogenic acid, ferulic acid, ellagic acid, gallic acid, and caffeic acid, followed by taxifolin, catechin, syringic acid, methyl gallate, vanillin, kaempferol, cinnamic acid and rutin. Among the results obtained, it was found that there was a difference in the concentration of phenolic compounds obtained using HPLC analysis from others in previous studies (Karthikeyan et al., 2012; Alaagib & Ayoub, 2015). This variation may be due to the method of plant cultivation and the surrounding environmental conditions, as well as the extraction methods of phenolic compounds.

3.2. GC–MS identification of SFE-oil

Supercritical fluid extraction (SFE) was utilized to extract oil from *Aucklandia costus* as a novel extraction technique. According to Kate et al. (2016), Wrona et al. (2017), Lammari et al. (2021) and other researchers, SFE is an optimum approach to obtaining a high oil yield and quality. The SFE-oil characterization profile is presented in Table 2 and Fig. S2. The results obtained from GC analysis led to the identification of

Table 1
HPLC analysis of *Aucklandia costus* phenolic extract.

Phenolic compounds	Concentration (μg/g)
Gallic acid	462.81
Chlorogenic acid	276.48
Coffeic acid	763.58
Syringic acid	32.97
Pyro catechol	148.29
Rutin	264.48
Coumaric acid	12.61
Vanillin	11.74
Naringenin	1809.59
Querectin	11.65
Cinnamic acid	14.51

Table 2
GC–MS profile of SFE-oil.

Retention Time (RT) (Min)	Name	Formula	Area (Abund. min)	Area Sum %
3.191	*trans*-Z-α-Bisabolene epoxide	C15H24O	75548.48	0.44
3.702	Nerolidol	C15H26O	59213.07	0.35
4.349	Pregnenolone, TMS derivative	C24H40O2Si	329179.13	1.93
4.42	3-Carene	C10H16	197909.79	1.16
4.472	Farnesol	C15H26O	107390.91	0.63
4.614	Patchulane	C15H26	28499.15	0.17
4.73	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	C15H24	43394.16	0.25
4.801	*cis*-Z-.alpha.-Bisabolene epoxide	C15H24O	361170.54	2.12
4.924	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	C18H26O	24425.6	0.14
5.086	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3β,17β)-	C22H32O2	17358.38	0.1
6.542	Squalene	C30H50	759075	4.45
6.613	Andrographolide	C20H30O5	63353.68	0.37
6.8	Methyl 10,12-pentacosadiynoate	C26H44O2	156125.56	0.91
6.981	Campesterol, TMS derivative	C31H56OSi	617092.92	3.62
7.188	Stigmasterol, TMS derivative	C32H56OSi	774458.96	4.54
7.68	β-Sitosterol, TMS derivative	C32H58OSi	12555797.38	73.56
8.075	1-Heptatriacotanol	C37H76O	585837.27	3.43
9.051	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-	C15H24O	197903.05	1.16
9.342	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z, E)-	C15H26O	115001.56	0.67

19 components in the SFE-oil. They were classified into ten chemical categories, sesquiterpenes, aliphatic, monoterpene hydrocarbons, ketone/aldehyde, aromatic monoterpenoids, alcohols, sesquiterpene oxide, sesquiterpenes lactone, aromatic sesquiterpene, and fatty acid ester/-sterols. The principal compound in SFE-oil was β-sitosterol as the major compound (73.56%), followed by stigmasterol (4.54%) and squalene (4.45%), whereas other compounds were identified in minor amounts. These findings differed from those of Deabes et al. (2021). They obtained 14 compounds from GC–MS analysis of the *S. costus* ethyl acetate extract. Butanedioic acid, the 2TMS derivative, recorded the highest percentage at 20.4%, followed by D-(-)- fructofuranose, pentakis(trimethylsilyl) ether (isomer 1), androstan-17-one, 3-ethyl-3-hydroxy-, (5. alpha)-, caffeic acid, the 3TMS derivative and L-(-)- sorbofuranose, pentakis (trimethylsilyl) ether with area percentages of 18.4%, 16.5%, 14.4%, and 11.4%, respectively. This variation may be due to the plant cultivation method, climate conditions, and extraction method used.

3.3. Characterization of nanophytosomes

The size distribution, polydispersity index and ζ-potential and encapsulation efficiency (EE) of SFE-oil and PE-loaded nanophytosomes are shown in Table 3. According to the results, a variation was found between SFE-oil and PE-loaded nanophytosomes in size values. The size distribution of PE-loaded nanophytosomes was 268.40, 202.73 and 168.47 nm higher for F1, F2 and F3, respectively ($P < 0$ 0.05), than SFE-oil-loaded nanophytosomes (155.80, 192.97 and 170.97 nm for F4, F5 and F6, respectively). Based on the PDI results, it was observed that the PDI was <0.50 (highly homogeneous size distribution), resulting in a high stability index for all formulas (El-Menshawe et al., 2018; Nazari et al., 2019).

The existence of phenolic extract in the phytosomes structure might explain this rise. Machado et al. (2019) revealed that phenolics are included in the aqueous phase of phytosomes during synthesis through reversed-phase evaporation, resulting in an increase in size distribution.

The ζ-potential values of SFE-oil-loaded nanophytosomes were higher by −15.10, −14.27 and −14.97 mV for F4, F5 and F6, respectively ($P < 0.05$), than those of PE-loaded nanophytosomes (−6.27, −20.13, −23.80, −20.17 mV for F1, F2, and F3, respectively). This result was probably because PE-loaded nanophytosomes with a positive charge (the phenol molecules have a partial positive charge on the oxygen atom due to resonance) interacted with the negatively charged phosphate group in phosphatidylcholine, giving the resulting nanophytosomes a reduced negative charge. In general, ζ-potential values of above or under ±30 mV were observed to prevent nanovesicles from clustering and decrease electrostatic force between nanophytosomes, which might contribute to the creation of large aggregates of nanophytosome particles (Xu et al., 2022).

To assess the effectiveness of nanocarriers in delivering active substances, EE is a critical factor in nanobased delivery systems (Rai et al., 2022). Table 3 shows the EE findings for PE- and SFE-oil-loaded nanophytosomes prepared using three different formulations, demonstrating the extraordinary capability of nanophytosomes as carriers. The developed nanophytosomes showed high EE values (Table 3) for both PE- and SFE-oil-loaded nanophytosomes. The developed systems' high EE values could be attributed to the employment of inner and outer polar functional sites of soybean lecithin for PE and residual phytosomes, in addition to lipophilic bilayer space. Nazari et al. (2019) investigated garlic essential oil (GEO) loaded in nanophytosomes as a new phytochemical delivery vehicle using three varied techniques and found that GEO nanophytosomes using homogenization-probe sonication (the same technique used in this study) demonstrated great promise with a size of 115 nm and EE of 86.00% (which is comparable to the values found for PE- and SFE-oil-loaded nanophytosomes).

3.3.1. Morphology

TEM was used to characterize the morphology of the PE- and SFE-oil-loaded nanophytosomes, which revealed spherical vesicles in all

Table 3
Size distribution (SD), polydispersity index (PDI), ζ-potential, and encapsulation efficiency (EE) of *Aucklandia costus* phenolic extract (PE) and supercritical fluid extraction of oil (SFE-oil)-loaded nanophytosomes.

Formulas	Size distribution (nm)	PDI	ζ-potential (mV)	EE (%)
Free nanophytosomes	110.63 ± 1.25^{d}	0.32 ± 0.00^{c}	-6.27 ± 1.35^{a}	–
F1	268.40 ± 10.01^{a}	0.46 ± 0.03^{ab}	-20.13 ± 0.42^{c}	89.67 ± 0.09^{b}
F2	202.73 ± 9.52^{b}	0.48 ± 0.10^{a}	-23.80 ± 0.72^{d}	78.94 ± 0.39^{c}
F3	168.47 ± 6.15^{c}	0.44 ± 0.06^{abc}	-20.17 ± 1.46^{c}	69.29 ± 0.36^{d}
F4	155.80 ± 18.94^{c}	0.35 ± 0.13^{bc}	-15.10 ± 1.06^{b}	92.09 ± 0.52^{a}
F5	192.97 ± 3.43^{b}	0.39 ± 0.03^{abc}	-14.87 ± 1.82^{b}	78.04 ± 2.09^{c}
F6	170.97 ± 2.41^{c}	0.36 ± 0.00^{bc}	-14.97 ± 0.87^{b}	70.75 ± 2.18^{d}

A similar superscript letter in every column indicates no statistically significant variation in values ($P<0.05$). The results are shown as the mean and standard deviation. F1 to F3: the ratio between PE and soy lecithin was 1:1, 1:2, and 1:3, respectively. F4 to F6: the ratio between SFE-oil and soy lecithin was 1:1, 1:2, and 1:3, respectively.

pictures (Fig. 1). Furthermore, the size distribution was heterogeneous, indicating the existence of certain aggregates, which is consistent with the PDI values reported (Table 3). The findings are consistent with other studies that have reported a size distribution greater than 200 nm for phytosomes used in the encapsulation of phenolic extracts (Direito et al., 2019; Singh et al., 2018) but less than 200 nm for phytosomes used in the encapsulation of oils (Nazari et al., 2019).

3.3.2. *In vitro digestibility*

Table 4 shows the changes in TPC and TFC content of digested PE- and SFE-oil-loaded nanophytosomes. According to the findings, the liberation of phenolics and flavonoids increased throughout digestion. It was stronger in the intestinal stage than in the gastric stage, suggesting that the phytosome encapsulation approach protects against digestive enzymes and pH changes through gastric digestion. The substantial preservation of phenolics and flavonoids through oral digestion might be ascribed to the brief duration of exposure (2 min), the limited action of α-amylase (Mosele et al., 2016), and the low solubility in salivary fluids (Ydjedd et al., 2017).

Similarly, phenolics and flavonoids were stable in the oral stage but rapidly degraded in the gastric stage (Chait et al., 2020). This significant reduction may be caused by the stomach fluid's lower pH (Moschona & Liakopoulou-Kyriakides, 2018). This could be explained by the strong electrostatic attraction between positively charged enzymes and negatively charged phenolic compounds at the pH of stomach fluid (Haslam et al., 2000; Ortega et al., 2011). As a result, fragile and vital phenolics and flavonoids may lose their bioavailability. Hence, preserving and managing their distribution at the end of the digestive process is critical. Flores et al. (2015) and Chait et al. (2020) found that the phenolic and flavonoid compounds released during the intestinal stage from the microencapsulated blueberry and carob pulp extracts were higher than after the gastric stage. These findings might be explained by the fact that encapsulating ingredients are weakly soluble at low pH (gastric fluid) compared to neutral pH (intestinal fluid). As a result, polyphenols are not susceptible to digestive enzymes or pH changes throughout the digestive process. Furthermore, during simulated intestinal digestion, the biotransformation of polymeric polyphenols to monomeric polyphenols enhanced free polyphenols in intestinal fluid (Mrduljaš et al., 2017). As a result, high polyphenol release was found at the end of the simulated digestion, indicating their improved digestibility. Table 4 outlines the phytosomes' protective impact on phenolics and flavonoids. During simulated gastrointestinal digestion, comparable results have been found for encapsulated mango peel (El-Messery et al., 2021a), carob pulp extract (Ydjedd et al., 2017), and blueberry extract (Flores et al., 2015). According to these investigations, TPC levels in the supernatant from stomach to intestinal digestion were increased. The current findings indicate that the lipid substances employed for phytosome stabilization influence phenolic compound sensitivity to digestive enzymes and pH at each stage (Saura-Calixto et al., 2007).

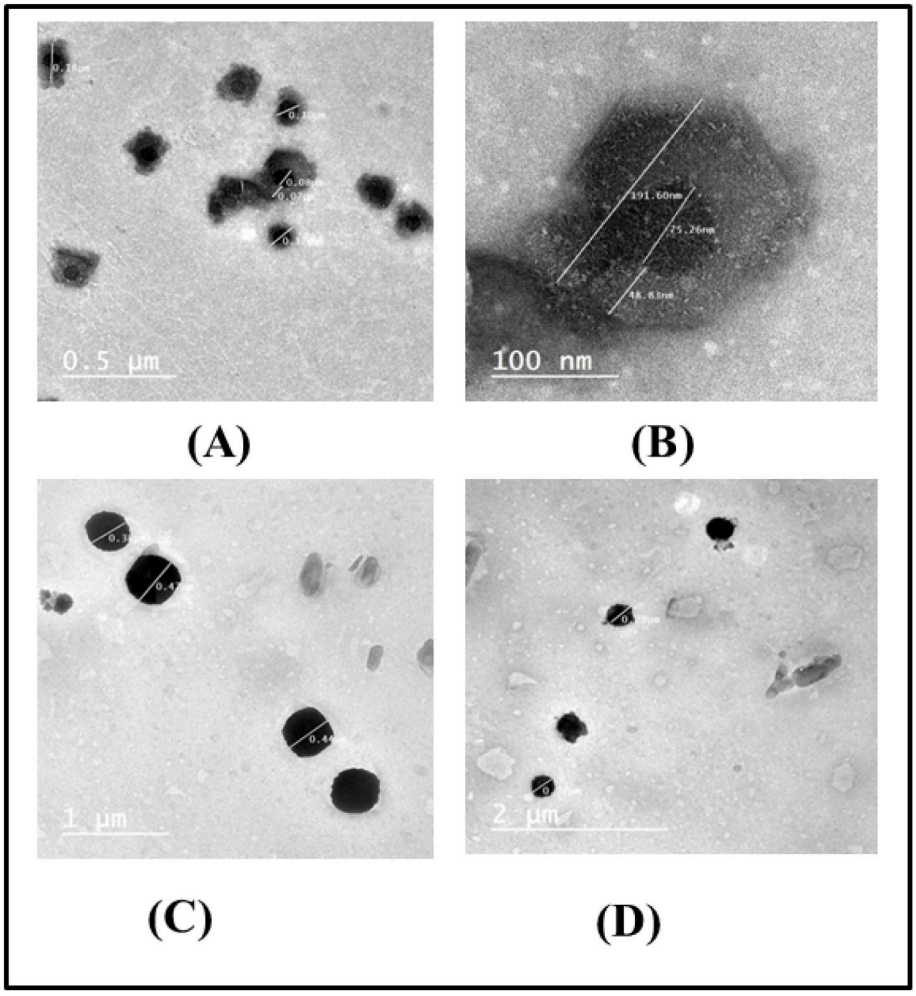

Fig. 1. Transmission electron microscopy (TEM) of *Aucklandia costus* phenolic extract (PE)-loaded nanophytosomes (a and b) and supercritical fluid extraction of oil (SFE-oil)-loaded nanophytosomes (c and d).

Table 4
The total phenolics (TP) and total flavonoids (TF) of *Aucklandia costus* phenolic extract (PE) and supercritical fluid extraction of oil (SFE-oil)-loaded nanophytosomes during *in vitro* digestion.

	Digested step	F1	F2	F3	F4	F5	F6
TPC (mg Gallic acid/100 g sample)	**Oral**	4.20 ± 0.28^{b}	5.13 ± 0.18^{a}	3.67 ± 0.24^{c}	2.71 ± 0.16^{d}	2.43 ± 0.04^{d}	3.55 ± 0.07^{c}
	Gastric	20.15 ± 0.21^{d}	30.50 ± 0.71^{b}	26.01 ± 0.01^{c}	17.44 ± 0.63^{e}	38.33 ± 0.46^{a}	12.42 ± 0.60^{f}
	Intestinal	205.49 ± 0.69^{b}	205.26 ± 0.37b	190.14 ± 0.20^{c}	180.21 ± 0.30^{d}	222.39 ± 0.55^{a}	173.31 ± 0.44^{e}
TFC (mg Rutin/100 g sample)	**Oral**	0.23 ± 0.05^{f}	0.41 ± 0.02^{e}	0.91 ± 0.02^{d}	1.01 ± 0.02^{c}	1.42 ± 0.04^{b}	1.10 ± 0.00^{a}
	Gastric	3.17 ± 0.24^{b}	4.08 ± 0.12^{a}	2.02 ± 0.02^{c}	2.06 ± 0.08^{c}	2.09 ± 0.12^{c}	1.62 ± 0.17^{d}
	Intestinal	40.14 ± 0.20^{d}	40.07 ± 0.10^{d}	42.40 ± 0.56^{c}	43.38 ± 0.54^{c}	56.45 ± 0.64^{b}	61.16 ± 0.23^{a}

A similar superscript letter in every row indicates no statistically significant variation in values ($P<0.05$). The results are shown as the mean and standard deviation. F1 to F3: the ratio between PE and soy lecithin was 1:1, 1:2 and 1:3, respectively. F4 to F6: the ratio between SFE-oil and soy lecithin was 1:1, 1:2 and 1:3, respectively.

3.4. Physical characteristics of stirred yoghurt

The pH and acidity values of all yoghurt samples were evaluated over 15 days of storage at 4 °C. Table 5 shows no significant variation between the control yoghurt and yoghurt fortified with MF-loaded nanophytosomes (T1, T2 and T3). At the end of storage, the pH values of all yoghurt samples declined from approximately 4.52 to 4.13, and the acidity values increased from approximately 0.95% to 1.09%, perhaps owing to the transconversion of sugar milk (lactose) into lactic acid by the starter bacteria. The reduced pH and increased acidity were substantially different for all samples obtained on days 7 and 15. The current findings agreed with Ghorbanzade et al. (2017), who found that the pH value of control yoghurt was lower than that of yoghurt fortified with nanocapsules of fish oil (liquid), with no significant variation between yoghurt samples. Furthermore, Akgün et al. (2020) found that yoghurt containing sour cherry extract in liposomal capsules had a higher pH than control yoghurt.

Water holding capacity (WHC) becomes a critical physical criterion for assessing yoghurt quality. Table 5 shows the changes in WHC in all yoghurt samples after 15 days of storage at 4 °C. WHC was substantially impacted by storage duration ($P< 0.05$). The WHC values were significantly reduced at the end of storage (56.50%, 55.50%, and 55.50% for T1, T2, and T3, respectively) compared to zero time (69.00%, 60.00% and 59.50% for T1, T2, and T3, respectively). This might be attributed to a gradual decrease during storage, which could affect the casein micelle matrix, allowing more whey to be released. Other researchers have observed comparable findings of greater WHC initially followed by reduced WHC% (Salvador & Fiszman, 2004; Staffolo et al., 2004 and Aryana & McGrew, 2007). The WHC values decreased in yoghurt fortified with MF-loaded nanophytosomes (T1, T2, and T3) during storage compared to the control sample. Lecithin, an emulsifier provided through phytosomes, might absorb some water and reduce separation. Furthermore, increasing total solids leads to greater water holding capacity and network stability, which may minimize syneresis (Achanta et al., 2007). Similarly, Shaker et al. (2000) found that increasing the viscosity of yoghurt with high total solids content substantially influenced the hardness of yoghurt gel and the degree of WHC.

Table 5
pH, acidity% and water holding capacity (WHC) of stirred yoghurt fortified with MF-loaded nanophytosomes during storage.

Parameter	storage Day	Control	T1	T2	T3
pH	**0**	4.52 ± 0.02^{b}	4.53 ± 0.02^{ab}	4.54 ± 0.01^{ab}	4.58 ± 0.02^{a}
	7	4.48 ± 0.01^{b}	4.50 ± 0.01^{ab}	4.51 ± 0.01^{ab}	4.53 ± 0.01^{a}
	15	4.43 ± 0.01^{c}	4.47 ± 0.01^{b}	4.48 ± 0.01^{ab}	4.50 ± 0.01^{a}
Acidity %	**0**	0.95 ± 0.01^{a}	0.98 ± 0.04^{a}	0.93 ± 0.03^{ab}	0.87 ± 0.01^{b}
	7	1.07 ± 0.01^{a}	0.99 ± 0.01^{b}	0.94 ± 0.01^{c}	0.94 ± 0.02^{c}
	15	1.09 ± 0.01^{a}	1.01 ± 0.01^{b}	0.98 ± 0.01^{bc}	0.96 ± 0.01^{c}
WHC* %	**0**	69.00 ± 1.41^{a}	65.50 ± 0.71^{b}	58.25 ± 1.06^{c}	58.25 ± 0.35^{c}
	7	60.00 ± 0.00^{a}	60.00 ± 0.00^{a}	60.00 ± 0.00^{a}	56.50 ± 0.71^{b}
	15	59.50 ± 0.71^{a}	56.50 ± 0.71^{b}	55.50 ± 0.71^{b}	55.50 ± 0.71^{b}

A similar superscript letter in every row indicates no statistically significant variation in values ($P<0.05$). The results are shown as the mean and standard deviation. Control: plain yoghurt without nanophytosomes, T1: yoghurt enriched with 5% MF-loaded nanophytosomes; T2: yoghurt enriched with 10% MF-loaded nanophytosomes; T3: yoghurt enriched with 15% MF-loaded nanophytosomes.

3.4.1. Color measurements

Color is an important feature that can influence consumer food choices; thus, it may influence their choices. Table 6 illustrates the color characteristics (L*, a*, and b*) of yoghurt samples fortified with MF-loaded nanophytosomes. The findings demonstrated that yoghurts fortified with MF-loaded nanophytosomes had considerably lower a*, b*, and L* values than the control yoghurt. Overall, the L* value (lightness) of the control sample was significantly higher ($P< 0.05$) than that of yoghurt fortified with MF-loaded nanophytosomes, which displayed low lightness values. Regarding the a* parameter, despite substantial variations ($P< 0.05$) among all yogurt samples for any storage day, a general trend to greenish color was noted since all yoghurt samples had negative values. Canella et al. (2018) speculated that the coloration might be attributable to the natural riboflavin concentration in milk. MF-loaded nanophytosomes, in particular, demonstrated increased yellowness, resulting in the highest b* in fortified yoghurt compared to the control sample. As a result, fortification yoghurts with MF-loaded nanophytosomes showed significant impacts, as measured by L*, a*, and b*.

3.4.2. Apparent viscosity

As reported in Fig. 2, the apparent viscosity of yoghurt samples decreased with increasing shear rate, mentioning a shear thinning phenomenon. Yoghurt is classified as a pseudoplastic fluid. Because of the low shear rate, pseudoplastic fluid has a comparatively high apparent viscosity at the beginning of the flow. As the shear rate

Table 6
Change in color parameters of stirred yogurt fortified with MF-loaded nanophytosomes.

Samples	Color parameters		
	L*	a*	b*
Control	85.78 ± 0.11^{a}	-1.48 ± 0.02^{d}	8.48 ± 0.01^{c}
T1	85.08 ± 0.05^{b}	-1.36 ± 0.02^{c}	8.49 ± 0.01^{c}
T2	85.09 ± 0.11^{b}	-1.27 ± 0.02^{b}	9.26 ± 0.02^{b}
T3	83.31 ± 0.03^{c}	-1.16 ± 0.01^{a}	9.34 ± 0.02^{a}

A similar superscript letter in every column indicates no statistically significant variation in values ($P<0.05$). The results are shown as the mean and standard deviation. Control: plain yoghurt without nanophytosomes, T1: yoghurt enriched with 5% MF-loaded nanophytosomes; T2: yoghurt enriched with 10% MF-loaded nanophytosomes; T3: yoghurt enriched with 15% MF-loaded nanophytosomes.

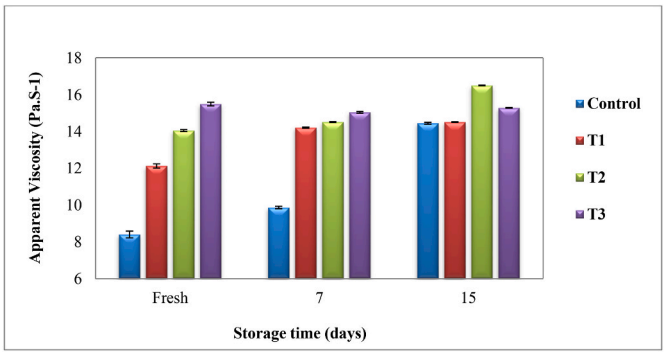

Fig. 2. Apparent viscosity of stirred yoghurt fortified with MF-loaded nanopphytosomes during storage. Control: plain yoghurt without nanophytosomes, T1: yoghurt enriched with 5% MF-loaded nanophytosomes; T2: yoghurt enriched with 10% MF-loaded nanophytosomes; T3: yoghurt enriched with 15% MF-loaded nanophytosomes.

increases, the slope of the curve steadily decreases, and the viscosity of the liquid decreases (Torres et al., 2018). Furthermore, over storage time, the apparent viscosity values of yoghurt fortified with MF-loaded nanophytosomes were higher than those of the control sample (Fig. 2). As a result, adding MF-loaded nanophytosomes to yogurt may improve its perceived viscosity. This finding was comparable to the findings of González Cuello et al. (2014). Furthermore, the high viscosity may protect the yoghurt matrix's structure and texture. As a result of its capacity to sustain viscosity during storage, MF-loaded nanophytosomes added to yoghurt encouraged superior viscosity behavior in comparison with the control, implying a potential use in food.

3.4.3. Sensory evaluation of yoghurts

Ten laboratory members evaluated the sensory characteristics of all yoghurts prepared. The sensory assessment parameters' mean scores were statistically examined, and the findings are shown in Table 7. There were substantial changes in aroma and flavor, according to the findings. Except for the control yoghurt, all yoghurt samples fortified with MF-loaded nanophytosomes differed in appearance, flavor, and texture (Table 7). According to the results, the addition of MF-loaded nanophytosomes to yoghurt showed no significant impact on texture in comparison with appearance and taste. Generally, all panelists rated the control and T1 samples as the best, with T3 receiving the lowest score. An earlier study found that nanoencapsulating fish oil with nanoliposomes had no significant influence on the overall acceptability of yoghurt samples during storage (Ghorbanzade et al., 2017).

4. Conclusion

Although research has shown that *Aucklandia costus* has a high richness in numerous nutrients, it has some disadvantages and downsides, including a foul odor, taste, being prone to oxidation, and a loose part of its activity within the digestive tract. As a result, numerous delivery strategies have recently been used to enhance the quality of *Aucklandia costus* and eliminate its restrictions. This research indicated that the supercritical fluid extraction and solvent-maceration techniques could be efficiently encapsulated by nanophytosomes with considerably high encapsulation efficiency and retention of high phenolics and flavonoids. Under simulated gastrointestinal settings, phytosome encapsulation offered a protective effect on PE and SFE-oil, displaying excellent free phenolic and flavonoid quantities at the end of the intestinal stage. PE and SFE-oil were delivered through yoghurt using a nanophytosome method, and the inclusion of encapsulated bioactive compounds enhanced the physicochemical qualities of the yoghurt. This study will help with the application of nanophytosome encapsulation to the fortification of food items, such as water-soluble and lipid-soluble compounds i.e. vitamins and carotenoids.

Table 7
Effect of MF-loaded nanophytosomes on the sensory characteristics of stirred yoghurt samples.

Samples	Appearance	Flavor	Texture	Overall acceptability
Control	9.40 ± 0.55^{a}	9.20 ± 0.84^{a}	9.20 ± 0.45^{a}	27.80 ± 1.64^{a}
T1	9.00 ± 0.71^{a}	8.40 ± 0.55^{ab}	7.80 ± 0.84^{b}	26.40 ± 0.84^{ab}
T2	8.80 ± 1.10^{a}	7.60 ± 1.34^{b}	6.60 ± 0.55^{c}	25.40 ± 1.73^{b}
T3	8.60 ± 1.52^{a}	7.20 ± 1.30^{b}	6.40 ± 0.55^{c}	24.80 ± 2.77^{b}

A similar superscript letter in every column indicates no statistically significant variation in values ($P<0.05$). The results are shown as the mean and standard deviation. Control: plain yoghurt without nanophytosomes, T1: yoghurt enriched with 5% MF-loaded nanophytosomes; T2: yoghurt enriched with 10% MF-loaded nanophytosomes; T3: yoghurt enriched with 15% MF-loaded nanophytosomes.

Authorship statement

Fadwa W. Abdulqahar: Investigation, Writing - Original Draft, Tamer M. El-Messery: Resources, Investigation, Conceptualization, Ahmed A. Zaky: Investigation, Formal analysis, Writing – review & editing. Marwa M. El-Said: Resources, Investigation, Writing - Original Draft.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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