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Nanoencapsulation of medicated ghee (*Guggulu tiktaka ghritam*) and its application in protein bar to enhance palatability

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Guggulu tiktaka gritham (GTG) is an ayurvedic formulation prepared using *ghee* (clarified butter). It is bitter with a disagreeable taste, and consuming high amounts as a single dose is a negative factor for oral administration. Nanoencapsulation of ayurvedic medicated *ghee* using a suitable water-soluble carrier is essential for absorption in the body as nanometer particles have a high surface area and can mask the bitterness. Its incorporation in a protein bar can reduce bitterness and enhance palatability. The oil-in-water emulsion technique was used to make *GTG* water-soluble, followed by ultrasonication to reduce particle size. The nanoemulsion prepared was spray-dried to obtain a powder and incorporated in the protein-rich pressed bar. Dynamic light scattering method and UV-VIS spectroscopy were used to ascertain particle size and confirm the stability of *GTG* after nanoencapsulation. The sensory profile of native *GTG*, nanoencapsulated *GTG* powder, and formulated protein bar were carried out. Nanoencapsulation of native *GTG* in a water-soluble carrier resulted in particle sizes averaging 397 nm. The addition of emulsifiers and salt decreased the particle sizes to 240 nm. Nanoencapsulation did not alter the chemical nature of native *GTG*, as evidenced by the absorbance observed in the 322-325 nm range. The sensory analysis showed that the nanoencapsulated *GTG*-incorporated protein bar was liked by 90% of the population. The challenges/bitterness related to oral administration of ayurvedic *GTG* formulation were alleviated by incorporating nanoencapsulated *GTG* into a protein bar, improving its palatability.

Keywords: *Guggulu tiktaka ghritam*, Medicated ghee, Nanoencapsulation, Palatability, Sensory analysis

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Ayurvedic system of medicine is prevalent in India, Sri Lanka, and other Asian countries; it is a traditional platform dating back to 5000 B.C. It also uses lipid-based formulations to treat several diseases. The formulations involve using concentrated decoctions/fine pastes of various herbs, which are later embedded into micro-structures of lipids. It has been reported that the hydrophilic bioactive components are in the nanometer particle size range (640 nm) once suspended in the vesicular micro-structure of anutailam (type of lipid)[1]. Ghrita is also an ayurvedic formulation prepared with *ghee* (clarified butter) along with a myriad of polyherbal decoctions[2,3]. *Guggulu tiktaka ghrita* (*GTG*) is a medicated ghee beneficial for skin ailments like psoriasis, eczema, dermatitis, and arthritic conditions (osteoarthritis, rheumatoid arthritis, cervical and lumbar spondylitis). It is used to heal wounds, abscesses, sinuses, and fistula. An excellent formula for all types of inflammations especially on connective tissues, bones, joints, and tumors. It is also beneficial in skin diseases of Vata-Kapha predominance. Effect on Tridosha - balances Vata and Kapha at the level of skin and joints, improves digestion and purifies blood[4-6].

The disagreeable or bitter taste of medication has been a major challenge for oral administration, which is the case with most Ayurveda medicines. 'Snehapana'- an oral administration of medicated ghee or oil, is an integral part of Panchakarma (detox treatment) in the Ayurveda system of medicine. Administration of bitter-medicated ghee or oil often leads to nausea, vomiting, and even rejection of treatment by patients. This has been a negative factor or a major hurdle in the Ayurveda system of medical practice, especially, Panchakarma clinical practice. Owing to the beneficiary properties of these formulations, it is inevitable to administer these medicines.

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In an attempt to overcome constraints such as water solubility, the absorption of Ayurvedic medicine, and improved palatability, a novel nanoencapsulation method can offer a promising solution. It is a process wherein small particles of core materials (lipophilic/hydrophilic) are encapsulated within a wall material (water/lipid-soluble) to form capsules or particles in the nanometer size range[7]. The encapsulation method can also be employed to mask the pungency of spice oils[8,9], and protect bioactive compounds (polyphenols, micronutrients, enzymes, and antioxidants) from degradation under different conditions such as pH and temperature. It can also be employed for the controlled release of core material at targeted sites[10]. The reduction in particle size to the nanometer range imparts unique biological properties[11]. Onwulata *et al.*[12] have encapsulated clarified butter in sucrose followed by double encapsulation in vegetable wax. Further, a cost estimate has been carried out by encapsulating clarified butter in all-purpose flour, modified corn starch, and sucrose[13]. In the present study, nanoencapsulation of *GTG* masked bitterness and provided an alternate clinical practice to administer Ayurvedic medicines orally. The nanoencapsulated powder was mixed with a high protein legume powder and other minor ingredients to prepare a protein-rich nanoencapsulated medicated ghee bar, showing its food applications.

Materials and Methods

GTG, a herbal formulation (Table 1) used in the present study, was procured from Arya Vaidya Sala, Kottakkal, Kerala, India. Sodium caseinate was procured from Clarion casein, Gujrat, India. Tween 80 (T80) and sodium chloride were purchased from Loba-Chemie, India. Purified (reverse osmosis) water was used for all the preparations. *Bengal gram* flour containing 21% protein was procured from a local market.

Encapsulation procedure

The 5% (w/w) of *GTG* was added to sodium caseinate (7% w/w) solution and homogenized for 5

Table 1 — Herbal ingredients of *Guggulu tiktaka ghritam*

Sl No	Official name	Botanical name	Part used	Form	Qty
1	Ghritam	Ghee	-	As it is	12.144 mL
2	Nimba	*Azadirachta indica*	St. Bk.	Dct.	3.542 g
3	Amrita	*Tinospora cordifolia*	St.	Dct.	3.542 g
4	Vrisha	*Justicia beddomei*	Rt.	Dct.	3.542 g
5	Patola	*Trichosanthes cucumerina*	Pl.	Dct.	3.542 g
6	Nidigdhika	*Solanum surattense*	Rt.	Dct.	3.542 g
7	Patha	*Cyclea peltata*	Rt.	Pst.	0.088 g
8	Vidanga	*Embelia ribes*	Sd	Pst.	0.088 g
9	Suradaru	*Cedrus deodara*	Ht Wd	Pst.	0.088 g
10	Gajopakulya	*Scindapsus officinalis*	Rt	Pst.	0.088 g
11	Yavakshara	Carbonate of Potash	-	Pst.	0.088 g
12	Souvarchala	Black Salt	-	Pst.	0.088 g
13	Nagara	*Zingiber officinale*	Rz	Pst.	0.088 g
14	Nisa	*Curcuma longa*	Rz	Pst.	0.088 g
15	Misi	*Anethum graveolens*	Sd	Pst.	0.088 g
16	Chavya	*Piper mullesua*	Rt	Pst.	0.088 g
17	Kushtha	*Saussurea paniculatus*	Rt	Pst.	0.088 g
18	Tejovati	*Celastrus paniculatus*	Sd	Pst.	0.088 g
19	Maricha	*Piper nigrum*	Fr	Pst.	0.088 g
20	Dipyaka	*Trachyspermum ammi*	Sd	Pst.	0.088 g
21	Vatsaka	*Wrightia antidysenterica*	Sd	Pst.	0.088 g
22	Agni	*Plumbago zeylanica*	Rt	Pst.	0.088 g
23	Rohini	*Neopicrorhiza scrophulariiflora*	Rt	Pst.	0.088 g
24	Arushkara	*Semecarpus anacardium*	Fr	Pst.	0.088 g
25	Vacha	*Acorus calamus*	Rz	Pst.	0.088 g
26	Kanamula	*Piper longum*(wild var)	Rt	Pst.	0.088 g
27	Manjishtha	*Rubia cordifolia*	Rt	Pst.	0.088 g
28	Ativisha	*Aconitum heterophyllum*	Rz	Pst.	0.088 g
29	Visha	*Aconitum ferox*	Rz	Pst.	0.088 g
30	Yavani	*Cuminum cyminum*	Sd	Pst.	0.088 g
31	Guggulu	*Commiphora mukul*	O.R	Pst.	1.771 g

min at 15,000 rpm (IKA T25 digital ULTRA-TURRAX) followed by ultrasonication (Sonics & Mat. Inc., Model VC-750)[14]. Seven formulations, referred to as NG, were prepared by keeping the amount of native *GTG* and sodium caseinate constant but varying the amounts of T80, sodium chloride, and tween 20 (Table 2). The emulsifier and ionic strength are important parameters that affect the emulsion stability and particle size. The emulsion was spray-dried at an inlet temperature of 105°C, an outlet temperature of 66°C, and an air pressure of 1.5 kg/cm^2 (SPD-P 111, Technosearch, India).

Characterization

Physico-chemical analysis

Moisture content and water activity (a_w)

The moisture content was measured as per the AOAC method[15]. 10 g of sample was taken in a previously weighed aluminum dish and kept in a hot air oven (Serwell Instruments, Bangalore, India) at 105±1°C for an hour followed by cooling at room temperature in a desiccator and weighed. The process was repeated (heating was carried out for half an hour) till the difference between the two readings was less than 1 mg. The difference in weight of the sample before and after drying and the empty aluminum dish was used to calculate the moisture content %. The a_w was recorded at 27°C using an electric hygrometer (A_w Sprint NOVASINA, Switzerland).

pH and specific gravity

pH was recorded with a handheld pH meter (HANNA Instruments, USA). Specific gravity was calculated as a ratio of the density of native *GTG* to water. The mass-to-volume ratio of native *GTG* was measured using a pycnometer.

Table 2 — Formulations prepared using *GTG*

Sl No	Sample name	Fixed parameters	Variations
1	NG1	GTG+Sodium caseinate	-
2	NG2	GTG+Sodium caseinate	Tween 80 at 0.5%
3	NG3	GTG+Sodium caseinate	Tween 80 at 0.05%
4	NG4	GTG+Sodium caseinate	Tween 80 at 0.5%+5% 0.2M NaCl
5	NG5	GTG+Sodium caseinate	Tween 80 at 0.5%+10% 0.2M NaCl
6	NG6	GTG+Sodium caseinate	Tween 80 at 0.5%+15% 0.2M NaCl
7	NG7	GTG+Sodium caseinate + maltodextrin	Tween 80 at 0.5%+10% 0.2M NaCl

Flow ability

The flow ability was calculated using the Carr Index as per the following formula[16].

$$\text{Carr Index} = (\rho_{tapped} - \rho_{bulk}) \times 100 / \rho_{tapped}$$

Where ρ_{tapped} and ρ_{bulk} are tapped and bulk density.

To calculate tapped density ~2 g of powder sample was added in a 10 mL graduated cylinder and compacted by vertically lifting and dropping the cylinder until no change was seen in the power level. To calculate bulk density same amount was taken in a cylinder, however, the powder was gently compacted.

Determination of peroxide value of ghee

The peroxide values (PV) of native *GTG* and nanoencapsulated *GTG* powder were determined by following the AOCS (1997) Cd 8-53 method.

Particle size analysis and its variation during storage

The mean particle size and polydispersity index (PDI) of the nanoencapsulated *GTG* powder formulations were measured using a Malvern zetasizer (Malvern Instruments Ltd, Malvern, UK). Each measurement was carried out in triplicate and mean values were reported. The prepared formulations were packed in high-density polyethylene pouches and stored in a desiccator filled with silica gel at room temperature (27±2°C; humidity 65%). These formulations were checked for a change in particle size on the 0th, 10th, 20^{th,} and 30th days due to agglomeration.

UV-VIS absorbance and encapsulation efficiency

The UV visible absorbance (UV-1800, Shimadzu) of native *GTG*, ghee (without bioactive) was recorded by dissolving 100 mg each in aqueous acetone (3 mL water and 27 mL acetone) from 300 to 600 nm using aqueous acetone as blank. Similarly, nanoencapsulated *GTG* powder (100 mg) was weighed and vortexed for 4 h in water, centrifuged at 1,20,000 g for 45 min, following which the supernatant was collected, diluted with acetone (in the same ratio as mentioned above) to record the spectrum from 300 to 600 nm. Encapsulation efficiency-The residue settled at the bottom was collected to determine the free *GTG* in the sample by dissolving it in aqueous acetone and comparing the absorbance (triplicates) with the standard graph of native *GTG*. The difference between the theoretical *GTG* incorporated amount and free *GTG* was used to check and calculate the encapsulation efficiency as per the protocol mentioned in Rao *et al*.[14].

Nanoencapsulated GTG incorporation in protein-rich pressed bar

Nanoencapsulated *GTG* powder was mixed with a roasted legume (Bengal gram) flour (17%) and other minor ingredients such as coconut fiber (15%) along with sweeteners and binders (16%) to prepare a protein-rich food bar. The bar contained 52% nanoencapsulated *GTG* powder.

Protein estimation

The protein content in the food bar was analyzed using the modified Lowry method, described by Hartree *et al.*[17]. The assay was carried out by diluting the 1 g food bar with H_2O and adding 0.9 mL of solution A [2 g L^{-1} potassium sodium tartrate ($KNaC_4H_4O_6{\cdot}4H_2O$) and 100 g L^{-1} sodium carbonate (Na_2CO_3) in 0.5 M NaOH] before incubating for 10 min at 50°C. The samples were cooled down to room temperature followed by the addition of 1 mL of solution B [0.2 g L^{-1} $KNaC_4H_4O_6{\cdot}4H_2O$ and 0.1 g L^{-1} copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) in 0.1 M NaOH] and left for 10 min. Finally, 3 mL of solution C [Folin–Ciocalteu phenol reagent in H_2O (1:16 *v*/*v*)] was added before incubation for 10 min at 50°C. A standard curve was made of bovine serum albumin (BSA; 0, 0.0625, 0.125, 0.25, 0.5, and 1 g L^{-1}) and absorbance was recorded at 650 nm.

Sensory evaluation for overall acceptability

Sensory analysis of native *GTG*, nanoencapsulated *GTG* powder, and protein-rich food bar was carried out in terms of color, texture, appearance, aroma, taste, and overall acceptability, with 20 semi-trained panelists (aged 24 to 50 years old) comprising of volunteer staff and students of Central Food Technological Research Institute, Mysore, India of both sexes (10 men and 10 women). A 9-point hedonic scale was used to rate the individual attributes numerically. Scores were assigned from 'like extremely' point 9 to 'dislike extremely' point 1[18]. The informed consent was taken from the participants. The analysis was carried out as per the ISO standards (4121 and 11136) and CSIR-CFTRI guidelines.

Results and Discussion

Physico-chemical analysis

Table 3 lists a_w, moisture content, pH, specific gravity, and flow ability of native *GTG* and nanoencapsulated *GTG* powder (as per applicability). Encapsulation did not alter the pH of the sample significantly. Moisture content and water activity are important aspects related to the storage of oils, fats, powders, food, etc. The adsorption of water by food materials, when stored at ambient conditions, can lead to their degradation and affect their texture, flavor, and flow properties. Further, low moisture content (<10%) and water activity (< 0.5) are essential to ensure the microbial safety of the products[19]. The moisture content and a_w of native *GTG* was higher (0.627±0.002, 11.77%) than the nanoencapsulated *GTG* powder. This could be due to the presence of concentrated decoctions/fine pastes of various herbs used in *GTG*. The moisture content in spray-dried nanoencapsulated *GTG* powder was 9.73% with a_w 0.429±0.001 ensuring its stability against microbial spoilage. Low moisture content and surface water were responsible for the high flowability of nanoencapsulated *GTG* powder. Nanoencapsulated *GTG* powder with a flowability of 24.6% indicated that it could be incorporated with other flours for food product development.

PV expressed as milli-equivalents (mEq) peroxide per 1 kg of fat extracted from the food, is an indication of the quality of oils and fats. In general, PV should be less than 10-20 mEq/kg fat to avoid rancidity[20]. PV of native *GTG* ranged between 0.08 and 0.1 mEq/kg, while that of powder ranged between 0.045 and 0.056 mEq/kg (Table 4), indicating that nanoencapsulation lowered the occurrence of *ghee*

Table 3 — Physico-chemical analysis of native *GTG* and nanoencapsulated *GTG* powder

S. No.	Parameter	Native *GTG*	Nanoencapsulated *GTG* powder
1	Water activity (a_w)	0.627±0.002	0.429±0.001
2	Moisture content@ 105°C	11.77%	9.73%
3	pH	6.0	6.72
4	Weight g/mL	0.96±0.002	NA
5	Specific gravity @27°C	1.09±0.005	NA
6	Flowability	NA	24.6%

*NA- Not applicable

Table 4 — Peroxide value (PV) of native *GTG* and nanoencapsulated *GTG* powder during storage

Storage period (days)	Peroxide value (mEq/kg)*	
	Native *GTG*	Nanoencapsulated *GTG* powder
15	0.08±0.005	0.046±0.002
30	0.08±0.01	0.046±0.001
45	0.09±0.01	0.048±0.002
60	0.09±0.02	0.051±0.005
75	0.10±0.005	0.055±0.001
90	0.10±0.01	0.056±0.001

*milli equivalents of active oxygen per kilogram rancidity (degradation). Naturally, ghee shows higher peroxide values[21] but native *GTG* being a medicated ghee has natural antioxidants, which could be the reason for its lower peroxide values.

Particle size and its variation during storage

The nanoencapsulation was carried out by employing the oil-in-water emulsion method. This involves the encapsulation of oil (core) by an inert carrier that forms a coating around it. The alternate high and low-pressure ultrasonic waves generate cavitation bubbles and strong hydrodynamic shear forces. This helps to disrupt and break the big particles into smaller ones. However, chemical compositions also play a critical role in achieving smaller particles. The seven formulations after spray-drying resulted in the formation of nanoencapsulated *GTG* powder with varied particle sizes. The particle size and PDI of nanoencapsulated *GTG* powder were measured on the 0^{th}, 10^{th}, 20^{th}, and 30^{th} day. Figure 1a shows the particle size of formulations on the 0^{th} day. It can be seen from the figure that the addition of emulsifiers and salt (to maintain ionic strength) reduced particle size. NG1 nanoencapsulated *GTG* showed an average particle size of 397 nm. The addition of T80 (0.05-0.5%) lowered particle sizes to 313-240 nm (NG2, NG3). The adsorption of T80 on the particles provides a steric repulsion that avoids the agglomeration of particles leading to smaller particle sizes. Further, increasing T80 did not reduce the particle size (data not shown). The effect of ionic strength on particle size was also analyzed. The addition of 0.2M NaCl solution at various percentages 5, 10, and 15 led to the formation of particles of size 250, 217, and 224 nm (NG4, NG5, NG6), respectively. The optimum amount of 0.2M NaCl in NG formulations was 10% as any further increase did not alter the size significantly. Size reduction was attributed to the presence of electrical charges on the surface of particles leading to an increase in repulsion among the particles[22]. The influence of polysaccharide, maltodextrin, on the the steric interaction among protein molecules was also studied. It was observed that the addition of maltodextrin reduced the size by many folds and 150 nm average particle sizes were obtained in sample NG7. The above changes in size were accompanied by a reduction in polydispersity in particles as evident from PDI that reduced from 0.495 to 0.252.

The stability of the prepared formulations concerning change in particle size was studied for 30 days Figure 1b. The particle size was recorded at an interval of 10 days. It is clear from the figure that NG7 did not show a significant change in size over 30 days of storage. It can be concluded that since the moisture uptake was minimal no agglomeration of particles was observed. Hence, this sample was chosen for further studies.

UV-VIS absorbance and encapsulation efficiency

Figure 2 shows the UV-VIS absorbance of native *GTG* and nanoencapsulated *GTG* powder. It is clear from the figure that λ_{max} (maximum absorbance at wavelength) of native *GTG* and nanoencapsulated *GTG* was at 322 and 325 nm, respectively. Hence, it can be concluded that no significant changes can be observed in UV-VIS absorbance after

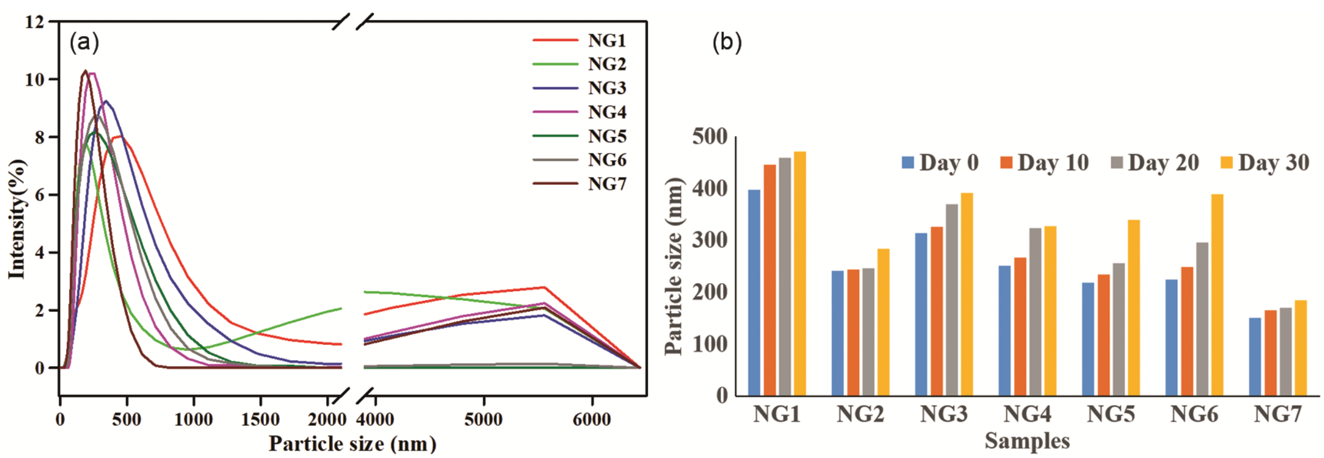

Fig. 1 — (a) Particle size of nanoencapsulated GTG formulations on 0^{th} day, (b) Variation in particle size of nanoencapsulated GTG formulations during storage of 24 days at 27±2°C

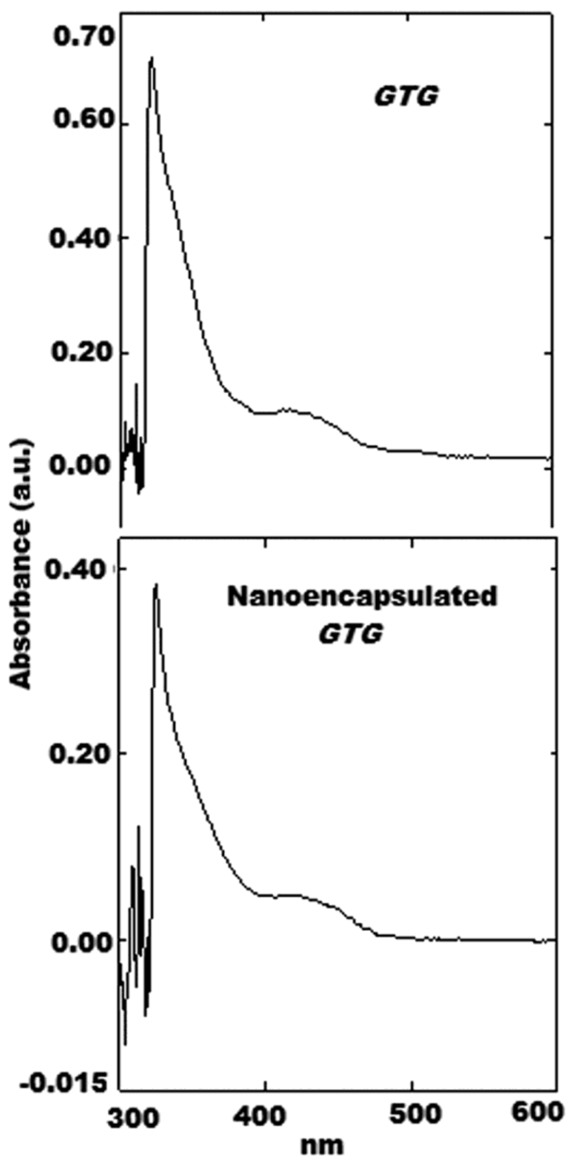

Fig. 2 — UV visible spectra of *GTG* and nanoencapsulated *GTG* powder

nanoencapsulation, indicating no alteration in the chemical composition of *GTG*. The *GTG* is an ayurvedic formulation consisting of many bioactive components (such as thymol, piperine, guggulsterone Z, lauric acid, linoleic acid, myristic acid, etc.)[23]. Therefore it was difficult to attribute the absorbance at this wavelength to a particular bioactive. However, a UV-VIS absorbance of *ghee* (devoid of bioactive) was compared (data not shown) which, also had λ_{max} at 321 nm although a broad hump in the region 415-475 nm was missing. The present work aimed to show the overall retention of *GTG* after nanoencapsulation using UV-VIS absorbance spectra and similar results were reported by Rao *et al.*[12] during their studies of nanoencapsulation of curcumin.

The encapsulation efficiency of the nanoencapsulated samples ranged from 65-87%. The encapsulation efficiency of the samples NG1, NG2, NG3, NG4, NG5, NG6, and NG7 was 65%, 73%, 68%, 78%, 80%, 80%, and 85%, respectively. This indicates that the role and amount of surfactant, ionic strength, and polysaccharide were crucial in enhancing the encapsulation efficiency of the samples.

Sensory evaluation for overall acceptability

The native *GTG* is extremely bitter and needs to be altered to make it palatable. The major components of *GTG* were *Azadirachta indica*, *Tinospora cordifolia*, *Justicia beddomei*, *Trichosanthes cucumerina*, and *Solanum surattense*, which, contribute to the bitterness, and make it unpalatable. In an attempt to reduce this bitterness and improve its palatability a protein bar Figure 3a was formulated. Sensory analysis of both native *GTG* and nanoencapsulated *GTG* powder was carried out. Figure 3b shows that native *GTG* was dark brown while the encapsulated powder cream to light beige. Further, the native *GTG* had a ghee-like aroma and taste while the powder was milky due to the wall material (sodium caseinate) used for encapsulation. Herbal notes and bitterness were dominant in both products but higher in native *GTG*. Bitterness is reduced marginally in encapsulated powder. The major change was that of an oily feel, characteristic of any ghee or ghee-like product, this was drastically reduced due to encapsulation. The overall acceptability was higher (6.5) for encapsulated ghee powder than the native *GTG* (2.5) thus the former was used to prepare a food bar.

A food bar prepared with added legume flour and coconut fiber made it more acceptable in terms of taste by reducing the bitterness. Sensory evaluation is quintessential to obtaining a complete analysis of various properties of food as perceived by the human sense[24]. Sensory evaluation of the food bar was carried out in terms of color, aroma, taste, texture, and OAA on a 9-point hedonic scale. The OAA scores are shown in Figure 3c and the food bar had an OAA of 8.2±0.31. An attempt was made to reduce the bitterness and enhance the sensory appeal of *GTG*. Further, the protein content was calculated to be 30 g/100 g of the pressed bar.

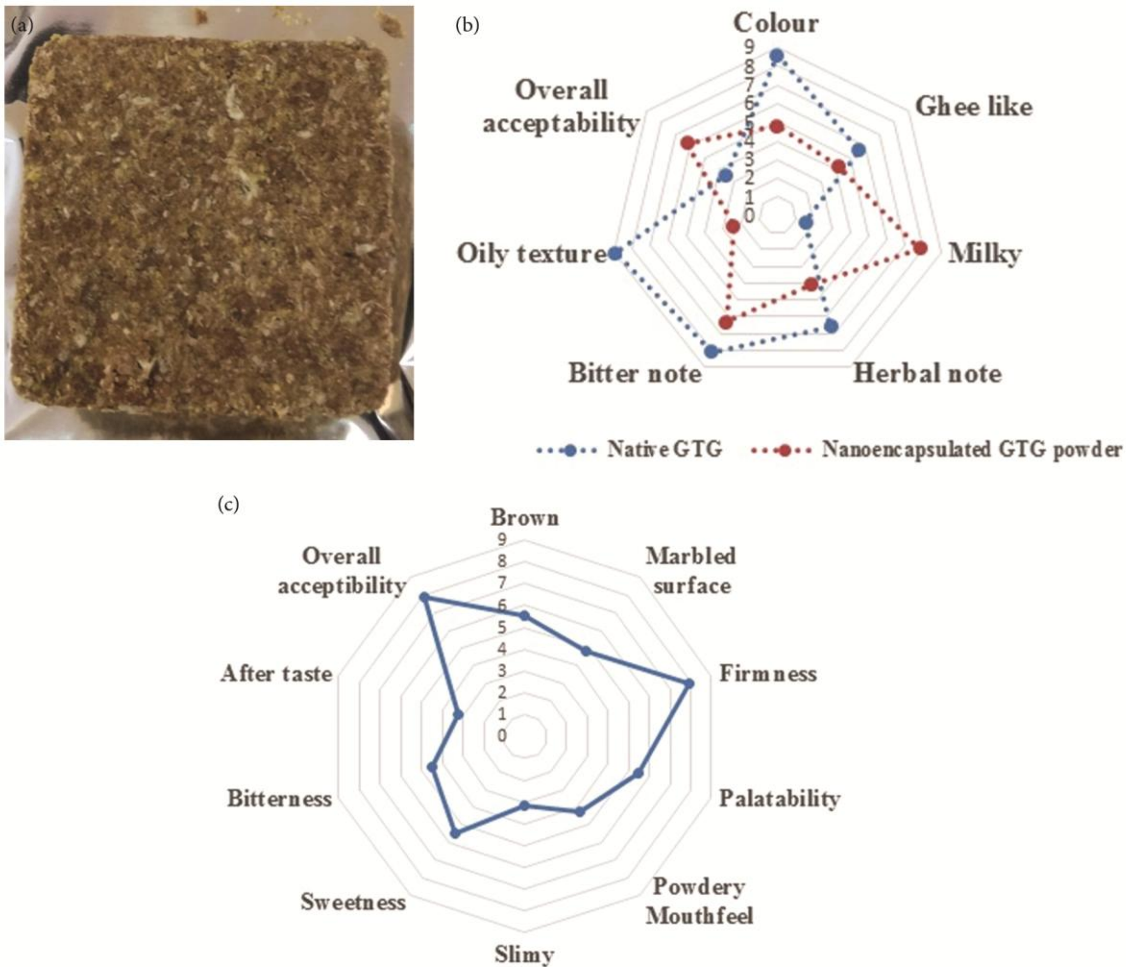

Fig. 3 — (a) Protein bar with encapsulated *GTG* powder, (b) Sensory profile of *GTG* and nanoencapsulated *GTG* powder, and (c) Nanoencapsulated *GTG* powder incorporated protein-rich pressed bar

Conclusions

The present work showed that the nanoencapsulation of the *Guggulu tiktaka gritham* (*GTG*) could mask the its bitterness and unpleasant taste. The protein bar prepared from nanoencapsulated *GTG* (particle size of 150 nm) showed good palatability and OAA of 8.2. The basic compositional property of *GTG* was confirmed with UV-VIS at a λ_{max} of 321 nm. The influence of surfactant Tween 80, ionic strength, and maltodextrin was studied. Further, studies are underway to ensure the medication compliance of *GTG* concerning dosage concentration and compare the clinical data with conventional dosage forms in the management of dermatological disorders (psoriasis and eczema).

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Conflicts of Interest

The authors declare that there is no conflict of interest.

Author Contributions

SR contributed for data curation and formal analysis. KPN initiated the conceptualization and Funding acquisition. CR contributed for in the product development and characterization. PJR contributed in writing the original draft, reviewing, editing the maunscript, and validation of results.

Informed Consent

The informed consent was taken from the participants.

Ethics Approval

The analysis was carried out as per the ISO standards (4121 and 11136) and CSIR- CFTRI guidelines.

Data Availability

The data will be made available on request.

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