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Sustainability Spotlight Statement

The sustainable approaches for preserving the bioactive compounds of edible flowers is by incorporating the flower's extract in food formulation which can provide better nutrients and various health benefits. The innovative aspect of this research lies in the encapsulation of Nongmangkha (*Phlogacanthus thyrsiflorus*) flower extract and utilized it for the development of functional food products with enhanced stability, bioactive retention etc. This approach addresses key challenges such as the low stability, poor bioavailability, and potential sensory issues of direct plant extract incorporation, offering a novel solution for fortifying foods like gummies and beverages with edible flowers.

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Functional food formulation by incorporating encapsulated extract of *Phlogacanthus thyrsiflorus* flower

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Abstract

Phlogacanthus thyrsiflorus flower (locally known as Nongmangkha), an underutilized medicinal flower, is a rich source of polyphenols with reported antioxidant, hypoglycemic, and hepatoprotective activities. However, its direct incorporation into food systems is constrained by sensory interference, thermal degradation of polyphenols, and limited bioavailability. This study presents a technological strategy for delivering phenolics of flower through encapsulation and their incorporation into watermelon gummies (0.5–2%) and ash gourd ready-to-serve (RTS) beverages (1%), with appropriate controls. The flower extract was obtained via ultrasound–microwave-assisted extraction (250 W, 15 min; 700 W, 5 min) and encapsulated through ionic gelation (3% sodium alginate and 5% $CaCl_2$). Total phenolic content in gummies increased (0.078 to 1.35 mg GAE/g), accompanied by improved flavonoid content and antioxidant activity, without altering some parameters such as moisture, titratable acidity (0.05–0.06%), pH (4.2–4.6), and total soluble solids (~72 °Brix) in gummies. Texture analysis revealed formulation dependent changes in hardness and chewiness, while 1.5% incorporation achieved optimal sensory acceptability. RTS beverages also demonstrated significantly elevated phenolic content and antioxidant radical scavenging capacity. Cell viability study of the flower extract exhibited minimal cytotoxicity in HEK-293 cell line at 12.5 µg/mL concentration. This study demonstrates a scalable approach for integrating encapsulated edible flower bioactives into functional foods formulation. Though in vivo bioavailability and storage stability warrant further investigation.

Keywords: Edible flower, extraction, cytotoxicity, gummies, ready to serve beverages

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1. Introduction

The growing interest in functional food products has paved a way for development of innovative food products with the aim to provide health benefits beyond basic nutritional attributes. Consumers generally seek functional food comprising enhanced sensory attributes such as aroma, flavor, texture, and visual appeal and also having similarities to traditional foods found in the market.[1] Functional foods are natural or processed foods that contain biologically active compounds. When it is consumed (nontoxic or adequate amount) provides clinically proven health benefits. The promotion of better health benefits, reduction of the risk of diseases (chronic, viral etc.) and management of their symptoms can be clinically evidenced by using biomarkers.[2] Functional foods bridge the gap between traditional or general food system and nutrition by delivering nutritional and bioactive compounds above normal nutrition, mitigating disease, promoting health and reducing health care costs etc.

One of the promising approaches involves incorporating bioactive compounds rich extracts for the development of food formulation which can provide better nutrients and various health benefits.[3] In contrast, direct use of phytochemicals may face various challenges as these substances are sensitive such as instability under some environmental factors such as heat, light, and oxygen, as well as limited bioavailability and may affect the color and taste of the developed food.[4] In this case, encapsulation of the flower extract is an effective approach to address these limitations prior to addition in food formulations. Encapsulation is a technique which encloses a core i.e., bioactive compounds within a wall material, enhancing their stability and controlled release while maintaining the extract's properties. This technique helps the development of food products with a better shelf life, higher nutritional value, and better sensory attributes etc.

Phlogacanthus thyrsiflorus flower also known as Nongmangkha is a red brick colored flower belong to acanthaceae family and an important medicinal plant that blooms from February to April. These flowers were reported to deliver beneficial health effects on hyperlipidemia as were showed antioxidant and radical scavenging activities and possessed hypoglycemic and hypolipidemic properties. This flower were believed to cure pox; prevent skin diseases like sores, scabies, have anti-allergic effects, treat wounds and tumors, act as a blood purifier, kidney stones, and liver disorders. The flowers contain steroids, terpenoids, flavonoids, phenols, etc. [5]

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Functional food products are described as offering additional health benefits beyond their conventional nutritional value.[6] Importantly, while developing functional food products, they must be standardized to ensure the safety of administering bioactive compounds as a health optimization tool. Indeed, it is crucial to set up a standardized process for the development of functional food products to guarantee the safe delivery of bioactive compounds to support improvement of health.[2] As functional food products demands are increasing which might be due to the rising costs of health care, the steady increase in life expectancy and the interest of the elderly in the improvement of life quality etc. Edible flowers can be termed as functional food due to its nutritional properties, antioxidant activity, antimicrobial activity, color, flavor, mood and stress reduction capability etc. Gummy candy is highly favored by all age circles. Gummy candies are produced using concentrated sugar solutions, gelling agents, and other components.[7] Gummies are generally low of required nutritional value and may lead to obesity, hyperglycemia and tooth problem etc.

The innovative aspect of this research lies in utilization of encapsulated flower extract for the development of functional food products. In this study encapsulated phytochemical extract from *Phlogacanthus thyrsiflorus* flower was incorporated into gummies and ready-to-serve (RTS) beverages and explored their physicochemical properties and sensory characteristics etc. Also, the cytotoxicity of flower extract was observed in this study.

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2. Materials and Methods

2.1. Materials

Flowers were collected from Sivasagar, Assam, India. Watermelon and Ash gourd were collected from local market Tezpur, Assam, India. All the materials or ingredients used for development of gummy and RTS beverage are pectin, agar-agar, citric acid etc. are food grades. All chemicals utilized for experimental analysis were analytical grade.

2.2. Collections of flowers

Flowers were harvested during their full bloom season in the morning hours. Flowers were procured in the morning (6 am to 10 am) and transported to laboratory on the same day and kept in refrigerator at 4 ℃. The collected flowers were then cleaned, trimmed, and washed with distilled water before drying them. Furthermore, the flowers were shade dried with blowing air (fan) and the dried flowers were ground, sieved and stored in an air tight container at refrigerated condition for further analysis.

2.3. Preparation of flower extract

Flower extract was prepared using an ultrasound-pretreated microwave-assisted extraction technique as standardized in our previous study.[8] Briefly, 1 g of shade-dried flower powder was mixed with 10 mL of 80% ethanol (solid-to-solvent ratio 1:10, w/v). The mixture was subjected to ultrasound pre-treatment at 250 W for 15 min to enhance cell wall disruption and facilitate solvent penetration. Subsequently, microwave-assisted extraction was performed at 700 W for 5 min to improve the recovery of bioactive compounds. The resulting extract was filtered through Whatman filter paper, and the solvent was removed using a rotary evaporator (ROTEVA-8703, Equitron, India) at 40 °C under reduced pressure. The dried extract was stored at 4 °C until further analysis and encapsulation.

2.4. Cytotoxicity assay

Crude extract of flower was diluted to 1 mg/mL with Milli-Q water and filtered by using 0.2 micron nylon filer. In this assay, HEK 293 cells (10,000 per well) were seeded into a 96-well plate. The cells were treated with extract concentrations ranging from 12.5 to 200 µg/mL for 24 h. After incubation, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL) was added and incubated for 3 h. Post-incubation, the media was removed carefully, and

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MTT dissolving solution was added. The absorbance was measured at a wavelength of 590 nm using UV-Vis spectrophotometer.

2.5. Preparation of encapsulates

The flower extract obtained through ultrasound-pretreated microwave-assisted extraction was subsequently used for encapsulation. Encapsulation was carried out using the ionic gelation technique, following the method described by Patel et al. [9] and Chetia et al.[10]. A 3% sodium alginate solution was prepared, and 3% of the dried flower extract (based on alginate weight) was dispersed uniformly in the alginate solution under continuous stirring to ensure homogeneity. Separately, a 5% calcium chloride solution was prepared at room temperature as the cross-linking medium. Encapsulation was performed using an encapsulator (Model B-390, Büchi, Switzerland), where the alginate–extract mixture was extruded drop wise into the calcium chloride solution under mild stirring to facilitate bead formation. The formed beads were allowed to cure in the calcium chloride solution for 30 min to ensure complete ionic crosslinking. Subsequently, the encapsulates were collected, washed gently with distilled water to remove excess calcium ions, and air-dried on glass Petri plates at ambient conditions. The dried encapsulates were packed in zip-lock pouches, placed in airtight containers, and stored at 4 °C until further use.

2.6. Gummy preparation

The composition of gummy was finalized by underwent various trials to process the gummy development. Initially, watermelon juice (50 mL) was taken and small amount of water is also added to mix well with other ingredients. With juice sugar (52.75%) was added and heated while stirring until it dissolved properly. Pectin (2.53%), agar-agar (2.11%) and citric acid (0.41%) was also added later into the mixture.[11] The whole mixture was mixed properly until it reached to TSS 72 °Brix. Except control (C) set of gummy, in other set of gummies 0.5%, 1%, 1.5% and 2% encapsulates were added and they are coded as G1, G2, G3 and G4 accordingly. The gummy mixture was poured into moulds immediately and kept in a refrigerator for setting properly. After the formation of gummies, they were kept in air tight container for further analysis (Fig. 1).

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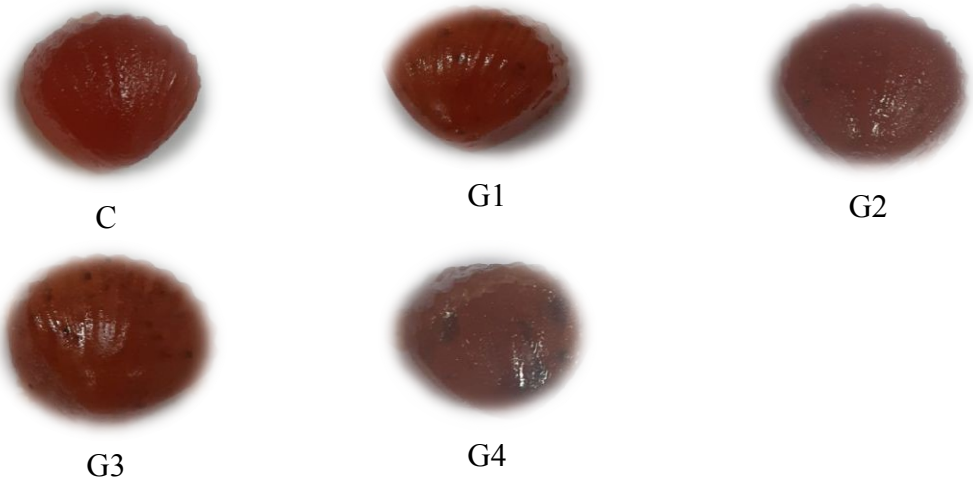

Fig. 1. Gummies prepared C, G1, G2, G3 and G4 with encapsulated beads.

C= Control gummy, G1= 0.5% encapsulates added, G2= 1% encapsulates added, G3= 1.5% encapsulates added and G4= 2% encapsulates added.

2.7. RTS beverage preparation

In case of RTS beverage, ash guard juice (50%) was taken and it was added to pre-prepared sugar syrup solution. The sugar syrup solution was prepared by dissolving sugar (11.5%) and citric acid (0.22%) in water (38.29%) with continuous stirring. The whole mixture was heated until it reached TSS 13 °Brix and homogenized with crude phytochemical extract (CERTS) and separately, another set was prepared where encapsulates were added (BRTS) after that in sterilized glass bottles the RTS beverage were filled hot and pasteurized it at 90 °C for 25 min. There were 3 different sets of beverages were prepared such as control (CRTS), with 1% encapsulates (BRTS) and with 0.5% of flower extract (CERTS). They were kept in refrigerator for further analysis.

2.8. Proximate analysis

The freshly prepared 5 sets of gummies which were coded as C, G1, G2, G3 and G4 analysed for moisture, total protein, total fiber and ash by adopting AOAC[12] method. Moisture content was determined by using drying oven (NDO-710W, Eyela) at 105 °C. Ash was analyzed by using muffle furnace. For fiber was analysed by employing Fibro plus (FES06) apparatus (Pelican Equipment, Chennai, India).

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2.9. Color analysis

The color parameter of all the gummies and RTS beverages were analyzed through hunter colorimeter (Hunter Lab, Reston, Virginia, USA). The color readings of L*, a* and b* parameters were recorded in triplicate for gummies (G1, G2, G3 and G4) and RTS beverages (CRTS, BRTS & CERTS).

2.10. Texture profile analysis (TPA)

The textural properties of gummies were determined by employing texture analyser (TA-XT plus, Stable Micro System, UK) using 0.5 R probe and the load cell was 30 kg. The texture parameters such as hardness, adhesiveness, cohesiveness and chewiness were analyzed for developed gummies (C, G1, G2, G3 and G4).

2.11. Titratable acidity

Titratable acidity of gummies and RTS beverage was determined by adopting volumetric method, where 1 g of gummy was dissolved in 9 mL of Milli-Q water. In case of RTS beverage, it was also mixed with Milli-Q water.[13] After that in that solution few drops of phenolphthalein were mixed. This homogenized solution was titrated with sodium hydroxide (0.1 N) and continue until there was purple color observed. With the help of Eq.1 the Titratable acidity (%) was measured.

$$\text{Titratable Acidity (\%)} = \frac{\text{Molarity of NaOH x mL of NaOH x equivalent weight of acid}}{\text{Weight of sample}} \times 100 \qquad (1)$$

2.12. TSS and pH

Total soluble solids were determined in gummies and RTS beverage. In a hand-held Atago refractometer, 2 mm thick slice sample (in case of gummies) were placed in the visor for the measurement.[14] In case of RTS beverage few drops of beverage were placed in the visor of refractometer and check the readings.

pH was measured by using a pH meter where it was equipped with a glass combined electrode. The gummies were cut into thin slices and mixed with hot water (1:10 w/v) at 27°C

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and it was homogenized.[13] In case of RTS beverage, it was taken to check the pH by using the pH meter.

2.13. Water activity

The water activity of gummies were measured by using water activity meter (Aqualab 4TE Decagon Devices Inc. Washington, USA) at 25 °C by placing 2 g of sample. [13]

2.14. Preparation of gummy extract for TPC, TFC and DPPH radical scavenging activity

The extraction of gummy was done by adopting the method of Mohd et al. [13] with a slight modification, by taking 5 g of Gummy dissolved in 50 mL of distilled water. It was kept for stirring in a magnetic stirrer until it was dissolved well. After that it was centrifuged at 6000 rpm for 15 min and the supernatant was filtered through Whatman no 1 filter paper. The extract was kept in refrigerated condition for further analysis.

2.15. Total phenolic content

The phenolic content of gummies and RTS beverage was determined by using Folin-Ciocalteu reagent (FCR) method.[15] 0.2 mL of extract of gummies and same amount of RTS beverage was taken in separate test tubes. It was mixed with 0.5mL of FCR (diluted with water 1:10) and after 5 minutes 2 mL sodium carbonate (20%) was added. The whole mixture was incubated for 1 h. The absorbance was measured at 650 nm by using UV-Vis spectrophotometer. Gallic acid used as a standard for the calculation of the calibration curve and the results were represented as mg gallic acid equivalent per gram (gummies) or mL (RTS beverage).

2.16. Total flavonoid content

Total flavonoid content of gummies and RTS beverage was done by following the method of Panhekar et al.[15]. 1mL extract was taken in a test tube and 4 mL distilled water was added into it. After that 0.3 mL of sodium nitrite (5%) was added into it and after 5 min 0.3 mL of aluminium chloride ($AlCl_3$) (10%) was added. The whole mixture was shaken and kept for 5 min and after that 2 mL of 1 M sodium hydroxide was added in to the mixture and finally it was diluted up to 10 mL by using distilled water and mixed well. The absorbance of the whole

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mixture was taken at 510 nm and results were expressed in terms of quercetin equivalent (mg QE/g).

2.17. DPPH radical scavenging activity

DPPH radical scavenging of gummies and RTS beverage was measured by adopting the procedure of Tundis et al.[16] with slight modifications. 0.5 mL extract was taken and it was mixed with 2.5 mL of DPPH solution (0.1 mM DPPH). This mixture was incubating for 30 min in dark at room temperature and wavelength was read at 517 nm by using spectrophotometer. DPPH radical scavenging activity was done by using the following Equation 2.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbanceof sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

2.18. Sensory evaluation

The developed gummies were subjected to sensory evaluation by approximately 30 semi-trained panellists (male and female; age group 25–35 years) from the Department of Food Engineering and Technology, Tezpur University. Panellists were selected based on their regular consumption of similar confectionery products and the absence of known food allergies related to the ingredients used in the study. Prior to evaluation, the purpose of the study and the evaluation procedure were explained to the participants.

Samples were coded with random numbers and presented in a randomized order to minimize bias. Sensory evaluation was conducted using a nine-point hedonic scale, where 1 represented “dislike extremely” and 9 represented “like extremely.” The panellists evaluated the samples for appearance, color, taste, mouthfeel, aftertaste, texture, and overall acceptability.

2.19. Statistical analysis

The data values were calculated for analysis of variance analysis (ANOVA) having level of significance at 95% ($p<0.05$) by using IBM SPSS Statistics v.20 (SPSS, Chicago, IL, USA). All the experiments for gummies and RTS beverage were carried out in triplicates and results are expressed as mean ± standard deviation. ANOVA was performed to determine significant

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differences among treatments. Duncan's Multiple Range Test (DMRT) was applied as a post hoc test to identify pair wise differences between means at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Cytotoxicity of flower extract in HEK-293 cells

To determine the cell viability, HEK-293 cells were treated with various concentrations (12.5 to 200 µg/mL) of the flower extract for 24 h. The extract exhibited appreciable cell vaibility (95.19 ±4.72%) at 12.5 µg/mL. Furthermore at 25 µg/mL, the cell viability was 87.93 ±1.11% (Table 1). Thus, the study indicates that at low doses the extract exhibits minimal cytotoxicity in HEK-293 cells.

Table 1.Cell viability of HEK-293 cells of ultrasound pretreated microwave assisted *Phlogacanthus thyrsiflorus* flower extract

Concentration of extract (µg/mL)	Cell viability (%)
0(Control)	100.00 ±0.00[a]
12.5	95.19 ±4.72[b]
25	87.93 ±1.11[c]
50	79.53 ±5.36[d]
100	70.33 ±4.17[e]
200	63.44 ±2.37[f]

3.2. Composition of gummies

The addition of encapsulated flower extract showed a significant enhancement in nutritional and functional properties of gummies as compared to the control gummy. Moisture (wet basis), ash, total fiber and total protein were analyzed for gummies and data is mentioned in Table 2. It was observed that the moisture content of all these gummies exhibited no significant differences ($p > 0.05$). This can be assumed that incorporation of encapsulates might not

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considerably alter the water retention capacity of the product. This consistency is desirable for maintaining textural properties and shelf stability. The lowest moisture content was observed in G1 gummy i.e.35.16% and highest was found in G3 gummy (37.18%). Burrey et al. [17] found moisture content of 55.08% in gummies containing sucralose was used as the substitute sugars and 29.44% in gummies where brown sugar was utilized. Teixeira-Lemos et al. [18] found the moisture content of 18.2 (g water/g sample) in Orange and honey gummy and 20.82 (g water/g sample) in Berries mix gummy. It can be understood that the moisture content of gummies varies based on their composition of the ingredients. The gummies showed to have intermediate level of moisture content due to its composition of high sugars along with other hygroscopic substances.

Ash content increased significantly in all functional gummies compared to the control gummy (0.1%). Encapsulates incorporated gummies showed to have ash content ranged from 0.66% (G4) to 0.76% (G1). This enhancement is likely due to the mineral content of the encapsulated phytochemical matrix and as well as overall encapsulates composition especially sodium alginate, demonstrating the nutritional enrichment of the gummies. The control recorded the lowest ash content (0.1%), whereas G1 exhibited the highest value (0.76%). Viswanathan et al.[19] studied compared ash content of sodium alginate from various brown seaweeds and they found that highest ash content of 23.01% was observed in *Padina gymnospora.* Others such as *Chnoospora implexa*, *Lobophora variegate* had showed ash content of 21.53%, 12.78% respectively. As encapsulates were made by using polymer sodium alginate, so it can be seen that sodium alginate also helped in the increment of ash content in gummies.

Total fiber content also exhibited a gradual increase from the control (0.29%) to encapsulates incorporated gummies. G4 (0.45%) showed a comparable fiber level to G3. The enhanced fiber levels are beneficial for functional food claims, as dietary fiber contributes to improved gut health. The crude fiber content showed a notable increase from the control (0.26) to G4 (0.45).

The Control had the lowest protein content (0.79%), while G3 recorded the highest value (1.32%). A slight decline in protein content was observed in G3 and G4 compared to G2, suggesting that the treatment process might affect protein stability or interaction with other

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components and also possibly due to variability in ingredient dispersion at lower encapsulates concentrations.

Table 2.Physicochemical properties of gummies

Parameters	Control	G1	G2	G3	G4
Moisture (% wet basis)	36.24±0.94[a]	37.26±1.83 [a]	37.97±1.32 [a]	37.85±0.69 [a]	37.04±1.73[a]
Water activity	0.74±0.00 [a]	0.78±0.00[b]	0.78±0.00[b]	0.79±0.03[b]	0.83±0.03[c]
Ash (%)	0.15±0.05[a]	0.68±0.5[b]	0.77±0.12[b]	0.77±0.08[b]	0.79±0.07[b]
Crude fiber (%)	0.26±0.04[a]	0.37±0.04[b]	0.37±0.07[b]	0.53±0.03[c]	0.57±0.02[c]
Protein (%)	0.74±0.06[a]	1.08±0.09[b]	1.38±0.16[c]	1.20±0.2[bc]	1.01±0.17[bc]
Titratable acidity (%)	0.05±0.03[a]	0.05±0.09 [a]	0.06±0.02 [a]	0.06±0.05 [a]	0.05±0.05 [a]
pH	4.33±0.25 [a]	4.3±0.2 [a]	4.6±0.15 [a]	4.2±0.25 [a]	4.3±0.25 [a]
Total soluble solids (°Brix)	72.17±0.29 [a]	71.83±0.29 [a]	72±0.0 [a]	72.17±0.29 [a]	72.17±0.29 [a]
TPC (mg GAE/g)	0.078±0.01[a]	0.089±0.009[a]	1.14±0.056[b]	1.20±0.031[b]	1.35±0.020[c]
TFC (mg QE/g)	0.015±0.002[a]	0.03±0.004[b]	0.041±0.007[bc]	0.048±0.003[cd]	0.053±0.03[d]
DPPH activity (%)	12.016±1.32[a]	12.92±0.33[ab]	13.83±0.27[b]	15.91±0.23[c]	16.35±0.40[c]

All data are the mean ± SD of three replicates. Mean followed by different letters in the same row differs significantly ($p \leq 0.05$)

G1=0.5% encapsulates added,G2=1% encapsulates added, G3=1.5% encapsulates added and G4=2% encapsulates added.

3.3. Water activity

Water activity was also in the range from 0.67_{aw} (C) to 0.78_{aw} (G3). Water activity showed a noticeable increase in bead-containing samples compared to the control. G1 (0.75), G2 (0.77), and G3 (0.78) exhibited significantly higher values ($p < 0.05$). This increase might be attributed to the moisture-binding capacity of the encapsulates. The slightly elevated water activity in of gummies compared to control gummy aligns with their increased bead concentration, which can influence microbial stability and shelf life. Water activity showed

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significant variation across the groups. The increase in water activity from G1 to G4 suggests a possible correlation with addition of encapsulates. Moisture of Gummy in generally is intermediate-level and as it is high in sugars and other hygroscopic substances, resulting in a low water activity.

3.4. Titratable Acidity, pH and TSS

There was no significant difference ($p > 0.05$) in titratable acidity (TA) and pH among all gummy formulations (Table 2). The TA values ranged between 0.05–0.06%, while pH values varied from 4.2 to 4.6, indicating that incorporation of encapsulated flower extract up to 2% did not significantly alter the overall acidity of the gummies. This stability may be attributed to the sodium alginate–calcium chloride ionic gelation system, which likely restricted direct interaction between the encapsulated phenolic compounds and the gummy matrix. In high-sugar gel systems, organic acids such as citric acid primarily govern the total acidity; therefore, the addition of encapsulated phytochemicals at low concentrations does not substantially influence the formulation. All samples retained the same TSS value of 72 °Brix, confirming that the inclusion of encapsulated encapsulates did not alter the sugar content or sweetness levels of the gummies. The total soluble solids (°Brix) showed no significant differences among the gummies, with values ranging from 71.83 to 72.17 °Brix.

Furthermore, our earlier findings demonstrated that encapsulation of flower extract using ionic gelation (3% sodium alginate and 5% $CaCl_2$) effectively protected phenolic compounds, as evidenced by the in vitro release study showing controlled release.[10] The encapsulation matrix provided protection against acidic degradation in simulated gastric conditions and prolonged phenolic availability during the intestinal phase

3.6. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

A significant increase in total phenolic content was observed from the Control (0.078 mg GAE/g) to G4 (1.35 mg GAE/g) (Table 2). The progressive rise in TPC with increasing the amount of encapsulates highlights the potential enhancement of phenolic compounds in Gummies. This trend highlights the contribution of encapsulated phytochemicals to the functional properties of the gummies.

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The total flavonoid content also increased significantly among these gummies. The control exhibited the lowest TFC (0.015 mg QE/g), while G4 recorded the highest value (0.053 mg QE/g). This trend aligns with the observed increase in phenolic content, reinforcing the contribution of flavonoid to the antioxidant profile of the treated samples and indicating that higher amount of encapsulates are necessary to achieve significant flavonoid enrichment.

Phenolic enrichment in functional food products has been widely reported when plant extracts are incorporated.[20] Earlier study found that jelly formulated with anthocyanin-loaded microcapsules exhibited enhanced physicochemical and functional properties compared to the control sample.[21] The substantial increase in TPC confirms that the alginate-based encapsulation system successfully protected and incorporated bioactive compounds without significant degradation during heating and gel setting.

3.7. DPPH Radical Scavenging Activity (%)

Antioxidant activity, measured as DPPH radical scavenging activity and it was observed to be increased significantly with increase in encapsulates concentration in Gummies from 12.016% (control) to 16.35% (G4) (Table 2). These results demonstrate the ability of encapsulates to deliver the bioactive compounds or phenolic compounds with antioxidant properties into the food matrix effectively. The correlation between TPC, TFC, and DPPH activity further underscores the role of phenolic and flavonoid compounds in enhancing antioxidant property. Binding properties of Sodium alginate may aid in the retention or activation of phenolic and flavonoid compounds, as reported by Huang et al.[22]. The progressive increase in TPC and TFC, along with improved DPPH radical scavenging activity, reflects potential of sodium alginate in enhancing antioxidant property.[23]

3.8. Color

The color parameters (L*, a*, b*) of the Gummies revealed significant differences among all the Gummies (Table 3). The lightness (L*) values decreased from 46.44 (Control) to 43.88 (G4), indicating a slight darkening of the Gummies. The redness (a*) values increased significantly in the gummies, with values ranging from 16.91 (Control) to 18.68 (G3), though there were no significant differences were observed among the Gummies incorporated with encapsulates.

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Yellowness (b*) showed a significant increase from 11.91 (Control) to 13.73 (G3), suggesting enhanced chromaticity in the treated samples. The observed changes in color parameters could be attributed to Maillard reactions and pigment incorporation resulting from the addition of colored encapsulates. The slight darkening and increased chromaticity observed are consistent with findings by Kim et al. [24], who attributed these changes to Maillard reactions and pigment transformation during processing or incorporation of encapsulates.

Table 3. Color parameters of gummies

Sample	L*	a*	b*
Control (C)	46.74±0.87^{c}	16.91±1.28^{a}	11.91±1.02^{a}
G1	45.82±0.48^{bc}	19.01±0.70^{b}	12.68±0.15^{ab}
G2	44.91±0.24^{ab}	19.74±0.17^{b}	13.40±0.27^{bc}
G3	44.87±0.11^{ab}	19.89±0.072^{b}	13.70±0.08^{c}
G4	43.88±1.08^{a}	18.68±0.88^{b}	13.73±0.35^{c}

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p \leq 0.05$)
G1= 0.5% encapsulates added, G2= 1% encapsulates added, G3= 1.5% encapsulates added and G4= 2% encapsulates added.

3.9. Texture profile analysis

Texture is an essential parameter to analyze for Gummies or any other confectionary items. Hardness, springiness, cohesiveness, gumminess and chewiness are measured for all the Gummies formulations and results are shown in Table 4. Hardness values varied significantly ($p<0.05$) across the Gummies. The Control Gummy exhibited a hardness of 36.21 g, which was higher than G3 (30.28 g) but lower than G1 (40.07 g). G4 had a value of 38.51 g, indicating that the addition of encapsulates affected the firmness of the samples. G2 showed intermediate hardness (34.84 g), suggesting a moderate effect of the treatment. Pyrovolou et al. [25] reported the hardness of commercial jelly gum was 43.1 (N), which is close to our results. Springiness decreased significantly from G1 (0.98) to G4 (0.56) Gummies, with the Control showing a value 0.93. G1 exhibited a relatively higher springiness, indicating potential variations in elasticity due to structural modifications from encapsulates. Springiness and cohesiveness showed a declining trend with increasing encapsulate incorporation, suggesting reduced elastic recovery and internal

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bonding strength. Incorporation of encapsulates into may disrupt matrix continuity and reduce elastic resilience. Cohesiveness helps to determine the strength, breakable range of inner bonds which hold the structure of Gummy and deformation.[26] Cohesiveness varied across the samples, with the highest value observed in G1 (0.08) and the lowest in G4 (0.029). The Control group displayed a moderate cohesiveness of 0.04. Gumminess and chewiness followed similar trends, as these parameters are dependent on both hardness and cohesiveness. Chewiness and gumminess are closely related to gummy hardness. The firmer the structure of the gummy, the harder it becomes, resulting in increased chewiness and gumminess.[27] Gumminess values followed a similar trend, with control recording the highest gumminess (11.39) and G3 the lowest (0.69). Chewiness decreased significantly ($p<0.05$) than the Control (10.59) in all the gummies.

Table 4. Texture profile analysis of gummies

Sample	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
Control(C)	36.21±0.44^{c}	0.931± 0.05^{c}	0.315±0.04^{c}	11.39±1.34^{c}	10.59±1.18^{c}
G1	40.07±0.32^{e}	0.975± 0.05^{c}	0.083± 0.01^{b}	3.34± 0.48^{b}	3.27± 0.63^{b}
G2	34.84±1.16^{b}	0.800±0.07^{b}	0.037±0.01^{a}	1.31±0.24^{a}	1.04±0.23^{a}
G3	30.28±0.54^{a}	0.706±0.05^{b}	0.030±0.01^{a}	0.98±0.24^{a}	0.69±0.17^{a}
G4	38.51±0.62^{d}	0.560±0.09^{a}	0.029±0.01^{a}	1.62±0.14^{a}	0.90±0.11^{a}

All data are the mean ± SD of three replicates. Mean followed by different letters in the same row differs significantly ($p \leq 0.05$)

G1= 0.5% encapsulates added, G2= 1% encapsulates added, G3= 1.5% encapsulates added and G4= 2% encapsulates added.

3.10. Sensory evaluation

Sensory evaluation is a scientific field that measures, examines, and interprets how people react or accept to certain food by their sense organs.[28] Sensory evaluation of food applies experimental design principles and statistical analysis. Sensory analysis of Gummies was done by within the 24 h of its preparation. As mentioned earlier 9 point hedonic scale was used with 1 being extremely disliked, 2 signified as slightly disliked, 3 was moderately disliked, 4 being

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slightly disliked, 5 being neither liked nor disliked, 6 was slightly liked, 7 was signified moderately liked, 8 was highly liked and 9 was extremely liked.[27] All the Gummies were evaluated by the parameters including appearance, color, taste, mouth feel, after taste, texture and overall acceptability with the help of semi trained panellist and results were shown in Fig. 2. The responses from panellist help to understand more insight and inferences bout the Gummies. In many ways, our perception of products is largely shaped by visual cues, especially color perception.[29] Since color significantly affects consumer appetite, it plays a crucial role in the overall palatability of food.[30] From Fig. 2 it can be observed that the appearance was mostly appreciated by panellist was control Gummy and after that G3. In case of color also Control Gummy C was highly accepted followed by Gummy G3. Similarly, taste was also highly appreciated by control Gummy C and followed by Gummy G3. Addition of encapsulates effect on the taste of the Gummies. Mouthful was found mostly accepted in case of control Gummy C and followed by G1 and G3. The after taste was found highly appreciated both in control Gummy C and G3. Texture was highly accepted in control Gummy C and followed by G2. Addition of encapsulates increase hardness of gummies which might not be like by the sensory panellists. The overall acceptability was observed in G3 and control Gummies. It can be assume that along with control Gummy also G3 Gummy was stood best consumer acceptability.

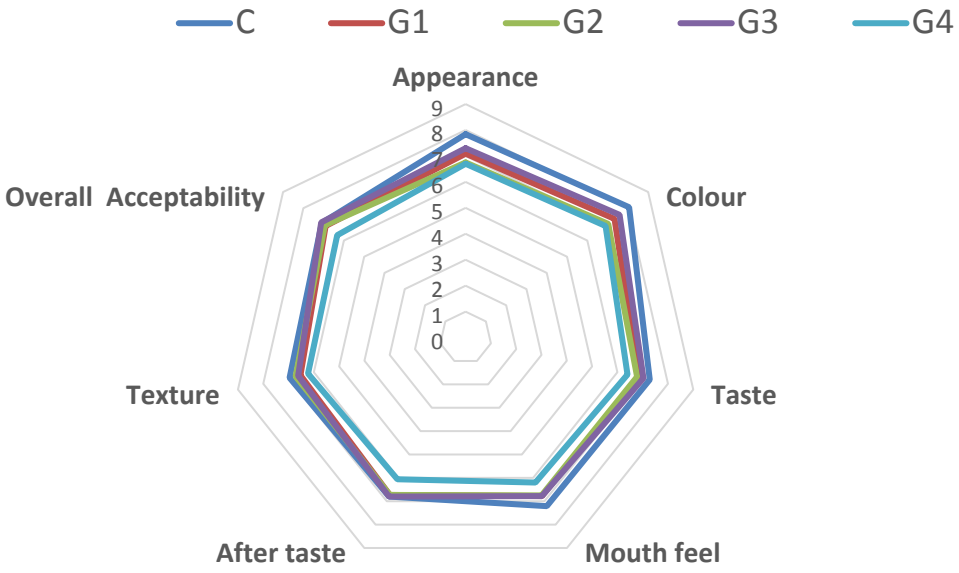

Fig. 2. Sensory score of Gummies.

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G1= 0.5% encapsulates added, G2= 1% encapsulates added, G3= 1.5% encapsulates added and G4= 2% encapsulates added.

3.11. Properties of RTS beverage

The results of the study on Ash gourd Ready-to-Serve (RTS) beverage formulations are presented in Table 5, which include physicochemical properties and color analysis of CRTS, CERTS, and BRTS. These parameters play a crucial role in determining the quality, stability, and consumer acceptability of RTS beverages. The physicochemical characteristics of the RTS formulations indicate significant variations among CRTS, CERTS, and BRTS in terms of Titratable acidity, pH, TPC, TFC, and DPPH radical scavenging activity.

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3.12. Total Phenolic Content, Total Flavonoid Content and DPPH radical scavenging activity

TPC content of CRTS, CERTS and BRTS were estimated and it was observed (Table 5) that addition of both encapsulates and crude significantly ($p < 0.05$) increase the TPC content as well as TFC and DPPH radical scavenging activity. The phenolic content of CERTS (3.81 mg GAE/mL) was significantly higher than BRTS (1.19 mg GAE/mL) and CRTS (0.41 mg/mL). Similarly, the flavonoid content followed the same trend (Table 5), with CERTS (0.64 mg QE/mL) exhibiting the highest TFC, followed by BRTS (0.49 mg QE/mL) and CRTS (0.07 mg QE/mL). Phenolic and flavonoid compounds contribute to antioxidant properties and have been linked to several health benefits, including reduced oxidative stress and improved cardiovascular health.[20] The higher phenolic and flavonoid content in CERTS could be attributed to the presence of certain bioactive compounds present in crude extract as well as in the encapsulates. The antioxidant activity (Table 5), as measured by DPPH radical scavenging percentage, was highest in CERTS (57.17%), followed by BRTS (51.43%) and CRTS (37.61%). The high antioxidant activity of CERTS may be directly related to its higher phenolic and flavonoid content. The high antioxidant activity of CERTS suggests that it may offer greater protection against oxidative stress, which is beneficial for health-conscious consumers. BRTS, with moderate antioxidant activity, remains a good alternative, whereas CRTS, with the lowest antioxidant capacity, is likely less effective in delivering health benefits. This suggests that CERTS might have better health-promoting properties, making it a more potent functional beverage.

3.13. Titratable acidity and pH

The acidity levels varied slightly, with CERTS showing (Table 5) the highest titratable acidity (0.31%), followed by CRTS (0.27%) and BRTS (0.27%). The pH values were also within a narrow range (3.23–3.7) (Table 5), with CERTS exhibiting the highest pH (3.7), suggesting a relatively lower acidity compared to CRTS and BRTS. The pH and acidity levels significantly affect the beverage's microbial stability and sensory properties.[31] A higher acidity level typically enhances the shelf life and contributes to a tart flavor, which can be desirable in fruit-based beverages. CERTS had slightly higher acidity, making it more stable and tangy.

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3.14. Color analysis

Color is an essential quality attribute of RTS beverages, influencing consumer preference and perception. CRTS exhibited the highest lightness value (31.57), while CERTS had the lowest (27.05) (Table 5). The incorporation of flower extract significantly affected the color attributes (L*, a*, b*) of the ash gourd RTS beverages compared to the control sample. A decrease in L* value indicates reduced lightness, which can be attributed to the natural pigments and phenolic compounds present in the flower extract. Plant-derived phenolics and flavonoids are known to contribute to darker tones due to their inherent chromophoric structures and potential oxidation reactions in aqueous systems. BRTS was intermediate at 29.06. Lower L* values suggest a darker appearance, which may be due to the presence of more intense pigments from the crude extract of flower. A lower L* value in CERTS suggests a more intense color, potentially due to the higher concentration of anthocyanins or carotenoids, which also contribute to its superior antioxidant properties. Earlier research reported of similar reductions in lightness have been reported in fruit- and herb-enriched functional beverages due to the addition of phenolic-rich extracts.[20] The a* values showed that CRTS (-0.42) and BRTS (-0.27) were more towards the green spectrum, whereas CERTS (0.9) had a slight red hue. A positive a* value in CERTS suggests that it contains higher anthocyanin or carotenoid pigments, which could also be linked to its higher phenolic content. The b* values revealed significant differences in the yellow-blue color range. BRTS (8.19) exhibited the highest b* value, indicating a more intense yellowish appearance, whereas CRTS had the lowest (2.86) and CERTS was in between (3.98). The variations in b* values suggest differences in ingredient composition, effect of processing conditions on encapsulates and crude extract of flower, and also pigment stability. The encapsulated extract exhibited comparatively moderated color changes relative to crude extract incorporation. This may be attributed to the protective alginate matrix, which can limit immediate pigment dispersion and reduce direct interaction with the beverage environment.

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Table 5. Physicochemical properties of RTS

Sl. no	Parameters	CRTS	CERTS	BRTS
1	Titratable acidity (%)	0.27±0.02[a]	0.31±0.08[a]	0.27±0.09[b]
2	pH	3.37±1.84[a]	3.7±1.47[a]	3.23±0.82[a]
3	TPC (mg/mL)	0.41±0.07[a]	3.81±0.15[c]	1.19±0.17[b]
4	TFC (mg/mL)	0.07±0.005[a]	0.64±0.008[c]	0.49±0.004[b]
5	DPPH activity (%)	37.61±1.85[a]	57.17±1.48[c]	51.43±3.2[b]
6	L*	31.57±2.45[a]	27.05±3.52[c]	29.06±2.85[b]
7	a*	-0.42±0.03[a]	0.9±0.07[c]	-0.27±0.02[b]
8	b*	2.86±0.24[a]	3.98±1.18[b]	8.19±2.09[c]

All data are the mean ± SD of three replicates. Mean followed by different letters in the same row differs significantly ($p \leq 0.05$)

4. Conclusions

The flower extract showed minimal cytotoxicity at 12.5 µg/mL in HEK-293 cells. While this observation is highly encouraging, future studies on appropriate animal models are required to ascertain the food safety parameters of this extract. In case of gummies, notable enhancements were observed in ash content, crude fiber, protein, and antioxidant activity, along with significant increases TPC, TFC and DPPH radical scavenging activity. However, variations in textural parameters such as hardness, springiness, and chewiness indicate that encapsulate incorporation influenced the structural properties of the gummies. In RTS beverages, the crude extract formulation (CERTS) exhibited higher phenolic and flavonoid contents and consequently stronger antioxidant activity compared to CRTS and BRTS. Future research should focus on optimizing formulation parameters, evaluating storage stability, and investigating in vitro and in vivo bioavailability to substantiate the health-promoting claims and support the commercial scalability of these functional food products.

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Conflicts of interest: The authors have declared that there is no conflict of interest for this work.

Ethical Review: This study does not involve any human or animal testing.

Availability of data and material: All data are presented in this article

Code availability: Not Applicable

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Author Contributions

IndraniChetia: Data curation, Formal analysis, Investigation; Writing-original draft;

Rituraj Chakraborty: Methodology, Resources to carryout Cytotoxicity Assay of flower extract.

RupakMukhopadhyay: Methodology and Resources to carryout Cytotoxicity Assay of flower extract.; Laxmikant S. Badwaik: Conceptualization, Project administration, Resources, Supervision, Writing-review & editing.

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Data Availability Statement

All data are presented in this article.