






## Development of Ginger Honey Candy Supplements with Propolis

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### ABSTRACT

The study aimed to develop a hard candy utilizing Vietnam's abundant honey supply, combined with ginger essential oil and propolis, to create a candy that enhances resistance. The candy formulation included sucrose, water, citric acid, glucose syrup, and honey, ginger essential oil and propolis, with honey concentrations tested at 10%, 15%, 20% and 30%. Sensory evaluation, conducted according to TCVN 3215–79 standard with 30 panelists, indicated the 10% honey formulation as the most preferred. Among propolis concentrations (0.5 – 2%), the candy containing 0.5% propolis was favored and used in subsequent formulations to determine the ginger essential oil level. Candies containing ginger essential oil (GEO) at 10, 20, 30, and 40  $\mu$ L, showing that candies with GEO had significantly higher total polyphenol content than other commercial candies. The results showed that the combination of honey, propolis and ginger essential oil enhances the antioxidant capacity of the candy. Therefore, the honey ginger candy supplemented with propolis has a distinctive color and flavor while retaining the inherent antioxidant properties of natural ingredients like propolis and ginger.

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## 1. Introduction

Honey has been used not only as a sweetener but also as a medicinal remedy in ancient cultures. It primarily consists of sugars such as fructose and glucose, along with amino acids, vitamins, trace elements, phenolic compounds, and flavonoids [1]. These phenolic compounds and flavonoids are particularly notable for their antioxidant and antibacterial properties [2]. Numerous studies have demonstrated honey's broad - spectrum antibacterial activity against both gram - negative and gram - positive bacteria, and it is currently used in modern medicine to treat infected wounds [2], [3].

In candy production, sucrose is the main raw material, but it poses health risks such as dental problems and diabetes, so honey can serve as a healthier alternative, acting as a sweetener while increasing the total phenolic content of the candy. Another valuable product from honeybees is propolis - a natural substance produced from plant secretions mixed with beeswax and pollen. The main ingredients of propolis include beeswax and fatty acids (20 – 35%), aromatic acids (about 10%), pollen (about 5%), and other ingredients such as minerals and vitamins, varying based on the type of pollen and bee species [4]. Propolis is a rich source of polyphenols, including various phenolic acids and flavonoids, which are responsible for its strong antibacterial activity [5]. Currently, propolis can be used in the production of pharmaceuticals and foods to help support health due to its antioxidant, antibacterial, antifungal, anti-inflammatory, antiviral, immunomodulatory, hepatoprotective properties, anti-allergic, infectious wound healing, anti-cancer and antidiabetic activities [6] – [16]. However, the strong odor and taste of propolis can limit its use in food products. Therefore, this study aims to develop hard candy from honey combined with ginger essential oil and propolis, to create a product that enhances resistance without compromising flavor and properties.

## 2. Materials and Methods

Honey and propolis (Tracybee Company, VietNam), ginger essential oil (Thuy Moc Viet Co. Ltd, Viet Nam), maltodextrin (Cong Thien Phat Company, VietNam), and glucose syrup (Bibica Corporation, VietNam) were used as ingredients for making hard candy.

### 2.1. Honey candy formulation

The candy was prepared by dissolving 70 g of sucrose in 30 mL of water and 20 g of glucose syrup [17]. The solution was stirred and heated to 145°C, then cooled to 100°C. At this stage, varying concentrations of honey (10%, 15%, 20%, 30% w/v), ginger essential oil (10 µL, 20 µL, 30 µL, 40 µL) and citric acid (0.3%) were added and stirred until homogeneous. Once the temperature reached 80°C, propolis was incorporated at concentrations of 0.5%, 1%, 1.5%, and 2% (w/v), followed by further mixing to ensure uniform distribution. The mixture was poured into a 55 – hole silicone mold (18.2 × 10.9 × 1.3 cm) and cooled at room temperature until it hardened. The candies were then wrapped and stored in a sealed jar for further analysis. In this study, the samples prepared with different concentrations of honey, ginger essential oil, and propolis are referred to as H, G, and P, respectively, throughout the text.

### 2.2. Sensory evaluation of products

Sensory evaluation was conducted using the scoring method according to TCVN 3215–79. A 20 – point scale was used, based on a uniform 6 - level, 5 - point scale (from 0 to 5). The sensory evaluation panel consisted of 30 members (who were previously given a few types of honey candies, propolis and ginger essential oil to familiarize themselves with color, odor, and taste). The samples were coded randomly, and panelists cleaned their palate with cold water before tasting each sample. Each panel member then assessed each individual criterion using a standard scoring sheet and the candy sample, assigning integer scores from 0 to 5. The average score for each sensory criterion was calculated from all panel members' evaluations, rounded to one decimal place. This average score was then multiplied by the corresponding importance coefficient for each criterion. For the ginger honey candy supplements with propolis, the importance coefficients for the criteria are as follows: hardness (0.5), color (0.6), odor (1.2) and taste (1.2), texture (0.5). Since the addition of propolis and ginger is intended to create a distinctive flavor in the product, the importance coefficient for odor and taste in this case is higher than for the other three factors. The total weighted score of all sensory criteria is then calculated to obtain the overall score.

### 2.3. Total polyphenols of honey candy

Gallic acid was used as a standard for quantifying total polyphenol content with diluted concentrations (25, 100, 200, 300, 400 mg/L). Candy extract was prepared by dissolving 1 g of candy in 10 mL of 10% methanol solution. The extract (0.1 mL) was then used to measure total polyphenol content using Folin-Ciocalteu reagent and absorbance at 735 nm. Each experiment was repeated three times. Total polyphenol content was determined using the following formula by Singh et al. (2017) [18]:

$$W = \frac{C \times V \times f}{m} \quad (1)$$

In there:

W: total phenolic content (mg GAE/kg).

C: Gallic acid concentration is determined based on the standard graph (mg/L).

V: volume of extraction obtained (mL).

m: mass of sample analyzed.

f: dilution factor of the extract.

### 2.4. Antioxidant capacity test

Two grams of candy were weighed and mixed with 5 mL of 80% ethanol. The mixture was centrifuged twice at 4500 rpm, 15 minutes and the supernatants were collected. DPPH radical

scavenging activity was measured by using the method of Baba et al. (2014) [19]: mixing 1 mL of DPPH with the extract (0.5 mL) and the absorbance at 517 nm was measured after 30 minutes in the dark.

$$\text{The \% inhibition was calculated using the following formula} = \frac{Ac - As}{Ac} \times 100 \quad (2)$$

Ac = Absorbance of the control at 517 nm. As = Absorbance of the sample at 517 nm.

### 2.5. Antimicrobial properties of honey candy

The antibacterial activity was tested on *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, and *Staphylococcus aureus* ATCC 25932, using the paper disk diffusion method. The bacteria strains were activated in 25 mL liquid Trypton Soya Broth medium (HiMedia, India), shaken at 180 rpm at 37°C for 24 hours. The OD at a wavelength of 600 nm and plate counting method were then used to determine a bacterial density. 100 µL of the bacterial suspension ( $10^8$  CFU/mL) was spread on a suitable nutrient media for each strain and then sterilized paper discs (6mm) impregnated with candy extracts (2.5 g candy dissolved in 1 mL of distilled water) were placed on it and incubated at 37°C. The results were read after 16 and 24 hours, by measuring the growth inhibition zone of the tested microorganisms. The distilled water and streptomycin were used as a negative and positive control, respectively. The experiment was repeated three times, and the diameter of the inhibition zone was determined by the formula:  $D(\text{mm}) = 2r$ , where (r) is the distance from the edge of the well to the bacterial growth circle [20].

### 2.6. Anti-inflammatory effect of formulated candy

Anti-inflammatory effect of candy extract was determined on lipopolysaccharide-stimulated RAW 264.7 macrophages, following the method described by Kim et al. (2021) [21]. The cells were cultured in a 96-well plate with Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum ( $2 \times 10^4$  cells per well) and incubated at 37°C in a 5% CO<sub>2</sub> incubator for 24 hours. Cell viability was evaluated using a colorimetric assay, where the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is reduced to its insoluble formazan, which exhibits a purple color and was measured at a wavelength of 540 nm using a cell imaging multi-mode plate reader (BioTek, Cytation 5, USA). The experimental setup included several controls and treatments to assess the anti-inflammatory effect of the candy extract on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. The negative control (NEG) consisted of cells treated with medium alone, without any inflammatory stimulus. The LPS control included cells stimulated with LPS but without any treatment. The positive control involved cells stimulated with LPS and treated with Dexamethasone (DEXAN), a known anti-inflammatory agent whereas the treatments involved cells were stimulated with LPS and treated with different concentrations of candy extract.

### 2.7. The physicochemical analysis of the ginger honey candy supplements with propolis

#### 2.7.1. Total soluble solid

Degree Brix is used as an indicator of the concentration of dissolved solids [22], which directly correlates with the sweetness. To check the Brix sugar concentration, weigh the candy (5, 10, 20 grams), place the candy sample in a glass cup, pour 40 mL of water into the glass cup of each sample, then heat the glass cup on the hot plate for 20 minutes at 60°C to dissolve the candy. Then, slowly add water to the glass cup until the volume reaches 100 mL and stir continuously until the solution is homogeneous [23]. Degree Brix was measured by a refractometer (Trans Instruments, Singapore) and the differences in Brix levels will be determined using a one-way ANOVA test with  $\alpha = 5\%$ .

#### 2.7.2. Moisture content

Moisture content of candy was estimated following TCVN 4069:2009, 3 g of the sample was dried in the drying oven (Daihan Labtech Co., Ltd, Korea) at  $105^\circ\text{C} \pm 2$  until a constant weight was achieved. It was cooled to room temperature in a dehumidifier before weight was determined.

$$\text{Moisture \%} = \frac{W2 - W3}{W2 - W1} \times 100\% \quad (3)$$

In there:

$W_1$  = Initial aluminum disc weight.  $W_2$  = Aluminum disc weight + initial sample weight.

$W_3$  = Aluminum disc weight + final sample weight.

### 2.7.3. Hardness and color measurement

The texture analysis using the texture analyzer (Lutron FR-5120, Taiwan) with the 2 mm cylindrical probe to measure the hardness of candy. The candies were placed on a secure base table and lowered the probe ~5 mm above the mold, ensuring proper alignment. When alignment is complete, the penetration test starts. The color of the candy sample is assessed using a handheld colorimeter (Konica Minolta, CR – 400, Japan). The results were expressed as CIE units: lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ).

All measurements were conducted in triplicate. The data are presented as mean  $\pm$  standard deviation (SD). Experimental data was statistically analyzed by one-way ANOVA ( $p < 0.05$ ) with the Minitab (version 21) software program.

## 3. Results and Discussion

### 3.1. Honey candy formulation

The sensory evaluation results indicate that as the honey ratio increases from 10% ( $H_{10}$ ) to 30% ( $H_{30}$ ), the hardness, odor, taste, and texture of the candy significantly decrease (Table 1), while the color becomes darker. This can be attributed to the increased moisture content in honey which inhibits sugar crystallization, leading to a less rigid structure. Additionally, the Maillard reaction, a chemical interaction between amino acids and reducing sugars, is known to cause browning in food products, explaining the observed color changes [24]. Statistical analysis indicates no significant difference between the sensory attributes of candies containing 10% ( $H_{10}$ ) and 15% ( $H_{15}$ ) honey. Therefore, the 10% honey formulation ( $H_{10}$ ) was chosen as the balance between quality and economic feasibility.

**Table 1.** Sensory evaluation results of honey candy

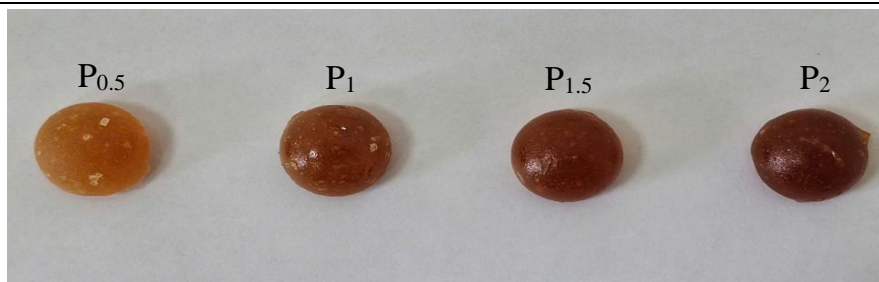
Samples	$H_{10}$	$H_{15}$	$H_{20}$	$H_{30}$
Hardness	$3.45 \pm 0.82^a$	$3.38 \pm 0.87^a$	$3.03 \pm 0.80^{ab}$	$2.58 \pm 0.93^b$
Color	$4.85 \pm 0.58^a$	$4.93 \pm 0.35^a$	$3.73 \pm 0.64^b$	$3.50 \pm 0.96^b$
Odor	$4.03 \pm 1.00^a$	$4.08 \pm 0.97^a$	$2.73 \pm 1.28^b$	$2.53 \pm 1.47^b$
Taste	$3.98 \pm 0.73^a$	$3.95 \pm 0.71^a$	$3.63 \pm 1.06^{ab}$	$3.35 \pm 0.98^b$
Texture	$3.48 \pm 1.15^a$	$3.33 \pm 1.23^{ab}$	$3.10 \pm 1.11^{ab}$	$2.73 \pm 1.26^b$
Overall	$19.78 \pm 2.35^a$	$19.65 \pm 2.33^a$	$16.20 \pm 2.62^b$	$14.68 \pm 2.97^c$

Values (Mean  $\pm$  Standard deviation) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey test,  $P < 0.05$ ). With  $H_{10}$  to  $H_{30}$  being the ratio of honey added.

**Table 2.** Sensory evaluation results of honey candy supplemented with propolis

Samples	$P_{0.5}$	$P_1$	$P_{1.5}$	$P_2$
Hardness	$4.00 \pm 0.87^a$	$2.80 \pm 1.13^b$	$2.73 \pm 1.02^b$	$3.07 \pm 0.98^b$
Color	$4.80 \pm 0.48^a$	$3.70 \pm 0.65^b$	$3.43 \pm 0.86^b$	$3.30 \pm 0.88^b$
Odor	$3.63 \pm 0.85^a$	$3.73 \pm 0.87^a$	$4.07 \pm 1.08^a$	$3.93 \pm 1.11^a$
Taste	$4.23 \pm 1.10^a$	$4.20 \pm 0.93^a$	$4.27 \pm 1.02^a$	$4.27 \pm 0.94^a$
Texture	$3.80 \pm 0.85^a$	$2.97 \pm 1.10^b$	$3.80 \pm 1.24^a$	$3.70 \pm 1.21^{ab}$
Overall	$20.47 \pm 2.60^a$	$17.40 \pm 2.67^b$	$18.30 \pm 3.68^b$	$18.27 \pm 3.25^b$

Values (Mean  $\pm$  Standard deviation) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey test,  $P < 0.05$ ). With  $P_{0.5}$  to  $P_2$  being the ratio of propolis added (%).



**Figure 1.** Color and external structure of candy when adding propolis (0.5-2%). With  $P_{0.5}$  to  $P_2$  being the ratio of ginger added.

In this experiment, propolis was incorporated at concentrations ranging from 0.5% to 2% (w/v), based on previous findings indicating that levels between 0.5% and 1.5% are effective in exhibiting antimicrobial and antioxidant activities [25]. The sensory evaluation results presented in Table 2 reveal significant differences in hardness, color, and texture among samples with varying propolis concentrations ( $P < 0.05$ ). The sample containing 0.5% propolis ( $P_{0.5}$ ) received the highest preference score for hardness ( $4.00 \pm 0.87$ ), which may be attributed to the reduction in candy hardness with increasing propolis concentration. Additionally, this sample exhibited the highest color preference score, indicating that panelists favored a lighter-colored propolis-enriched candy. Overall, the 0.5% propolis formulation was chosen as it provided a balance between structural integrity and sensory acceptability. This concentration was selected not only for its efficacy but also for its safety and consumer-friendly profile. According to established guidelines, the safe daily intake of propolis is 1.4 mg/kg of body weight [26], which corresponds to approximately 70 mg for an average adult. Each 3 g candy contains 15 mg of propolis and approximately 1.59 g of sugar. Therefore, consuming 3 to 4 candies per day would provide a total of 45 – 60 mg of propolis - well within the safety threshold - and a sugar intake of 4.77–6.36 g, which remains below the WHO's recommended limit of 25 g/day for free sugars (WHO, 2015) [27]. These findings support the 0.5% formulation as a safe and effective option that aligns with both functional and nutritional recommendations.

As shown in Table 3, the addition of ginger essential oil had no significant effect on the color, aroma, or texture of the candy. However, the  $G_{40}$  sample exhibited the lowest hardness value ( $2.90 \pm 1.10$ ) among the four formulations. The reduction in hardness observed in the sample with the highest ginger essential oil content may be attributed to the influence of ginger essential oil on sugar crystallization. Specifically, ginger essential oil has been reported to lower the temperature at which sugar begins to crystallize, thereby reducing the likelihood of crystallization at typical candy cooking temperatures and resulting in a softer texture [28]. Furthermore, the  $G_{40}$  sample received the lowest overall sensory score, which could be due to an excessively strong, spicy, or unpleasant taste, leading to decreased consumer acceptability [29]. There were no statistically significant differences in the sensory scores of tastes between the  $G_{10}$  and  $G_{20}$  samples. Based on these findings, the formulation containing 10  $\mu$ L of ginger essential oil was chosen based on the sensory test. However, to have better decision making on the final formula, the other following physicochemical characteristics were analyzed.

**Table 3.** Sensory evaluation results of honey propolis candy with different ginger oil concentrations

Samples	$G_{10}$	$G_{20}$	$G_{30}$	$G_{40}$
Hardness	$4.20 \pm 0.81^a$	$3.97 \pm 0.77^a$	$4.03 \pm 0.85^a$	$2.90 \pm 1.10^b$
Color	$4.83 \pm 0.38^a$	$4.77 \pm 0.50^a$	$4.70 \pm 0.60^a$	$4.67 \pm 0.71^a$
Odor	$4.00 \pm 0.83^a$	$3.53 \pm 1.04^a$	$3.60 \pm 1.25^a$	$3.67 \pm 1.21^a$
Taste	$4.33 \pm 1.06^a$	$3.83 \pm 1.09^{ab}$	$3.10 \pm 1.13^{bc}$	$2.70 \pm 1.26^c$
Texture	$3.77 \pm 1.14^a$	$3.83 \pm 1.05^a$	$3.77 \pm 1.25^a$	$3.57 \pm 1.36^a$
Overall	$21.13 \pm 2.85^a$	$19.93 \pm 2.69^a$	$19.20 \pm 2.49^b$	$17.50 \pm 3.37^b$

Values (Mean  $\pm$  Standard deviation) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey test,  $P < 0.05$ ). With  $G_{10}$  to  $G_{40}$  being the ratio of ginger added ( $\mu$ L).

### 3.2. Total polyphenols of honey candy

**Table 4.** Total polyphenol of honey propolis candy with different ginger oil concentrations

Samples	C <sub>1</sub>	C <sub>2</sub>	G <sub>10</sub>	G <sub>20</sub>	G <sub>30</sub>	G <sub>40</sub>
(mg GAE /kg)	115.0 ± 20 <sup>c</sup>	178.0 ± 77.8 <sup>c</sup>	340.9 ± 47.9 <sup>b</sup>	452.0 ± 32.6 <sup>b</sup>	461.3 ± 28.5 <sup>b</sup>	755.7 ± 37.8 <sup>a</sup>

Values (Mean ± Standard deviation) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey test,  $P < 0.05$ ). With C<sub>1</sub>, C<sub>2</sub> are 2 types of commercial candy. G<sub>10</sub> to G<sub>40</sub> being the ratio of ginger added.

The candy formulated with 10% honey and 0,5% of propolis supplemented with different amounts of ginger essential oil were used to evaluate the total polyphenol content and compared with two commercial candies that have honey in their ingredients. The results in Table 4 showed that the total polyphenol content of tested candies varied significantly. G<sub>40</sub> exhibited the highest polyphenol concentration (755.7 ± 37.8 mg GAE/kg), followed by G<sub>30</sub> and G<sub>20</sub>, while G<sub>10</sub> had the lowest (340.9 ± 47.5 mg GAE/kg). In contrast, commercial samples C<sub>1</sub> and C<sub>2</sub>, formulated with ginger jam and honey, exhibited significantly lower levels (Table 4). These findings indicate that the incorporation of propolis and ginger essential oil contributes to a substantial increase in polyphenol content, likely due to their naturally high phenolic composition and antioxidant properties [30]. In the study of Cedeno- Pinos et al (2021), jelly candies formulated with sugars exhibit limited antioxidant properties, but the addition of propolis dry extract (0.02%) significantly enhanced the antioxidant capacity without affecting the pH, color, or texture of the candies.

### 3.3. Antioxidant capacity test

Polyphenols are bioactive compounds that play a critical role in promoting health due to their exceptional antioxidant properties. They are renowned for their ability to neutralize free radicals, which are unstable molecules that can cause cellular damage and contribute to various chronic diseases. This is achieved through multiple mechanisms, including scavenging free radicals to prevent oxidative stress, donating hydrogen atoms to stabilize these reactive species, and serving as reducing agents that convert free radicals into less harmful molecules [31]. To evaluate the antioxidant potential of honey candies with propolis and ginger essential oil, DPPH radical scavenging activities were measured and compared to ascorbic acid. The results showed a significant difference ( $p < 0.05$ ) in antioxidant activity with the addition of 10 µL – 40 µL ginger essential oil and 0.5% propolis. The lowest IC<sub>50</sub> values were observed with 30 µL ginger essential oil and 0.5% propolis (111.05 ± 5.10<sup>c</sup>), but not significantly different with candy containing 40 µL ginger essential oil. The high polyphenol content in honey, propolis, and ginger essential oil likely contributes to the antioxidant capacity of the product.

**Table 5.** Antioxidant capacity analysis of honey propolis candy with different ginger oil concentrations

Samples	G <sub>10</sub>	G <sub>20</sub>	G <sub>30</sub>	G <sub>40</sub>
IC <sub>50</sub> (mg/kg)	137.09 ± 6.26 <sup>b</sup>	159.98 ± 3.25 <sup>a</sup>	111.05 ± 5.10 <sup>c</sup>	120.81 ± 9.74 <sup>bc</sup>

<sup>a</sup>All the values are calculated in triplicates (n=3). The values of the result represented in the form mean ± standard deviation. Values represented with different capital superscripts differ significantly in a row ( $p < 0.05$ ). With G<sub>10</sub> to G<sub>40</sub> being added the ratio of ginger.

### 3.4. Antimicrobial properties of honey candy

**Table 6.** Antimicrobial activity of honey propolis candy with different ginger oil concentrations

Samples	Zone of inhibition diameter (mm)		
	<i>Salmonella typhimurium</i> ATCC 14028	<i>Staphylococcus aureus</i> ATCC 25932	<i>E. coli</i> ATCC 25922
Streptomycin	16.17 ± 0.76 <sup>a</sup>	14.83 ± 2.02 <sup>a</sup>	15.50 ± 2.18 <sup>a</sup>
G <sub>10</sub>	2.67 ± 1.15 <sup>bc</sup>	3.00 ± 1.00 <sup>b</sup>	1.00 ± 1.73 <sup>b</sup>

G <sub>20</sub>	0.67 ± 1.15 <sup>c</sup>	1.67 ± 2.08 <sup>b</sup>	0.33 ± 0.58 <sup>b</sup>
G <sub>30</sub>	2.00 ± 1.00 <sup>bc</sup>	2.67 ± 0.58 <sup>b</sup>	2.00 ± 1.73 <sup>b</sup>
G <sub>40</sub>	4.00 ± 1.00 <sup>b</sup>	2.33 ± 0.58 <sup>b</sup>	4.33 ± 0.58 <sup>b</sup>

Values (Mean ± Standard deviation) with different superscripts in the same column are significantly different (one-way ANOVA and Tukey test,  $P < 0.05$ ). With G<sub>10</sub> to G<sub>40</sub> being added the ratio of ginger.

Table 6 presents the antimicrobial activity of the candy against *S. typhimurium*, *S. aureus*, and *E. coli*. Streptomycin, used as the positive control, exhibited strong inhibition against all tested strains, with inhibition zones exceeding 10 mm. In contrast, honey candy samples with propolis and ginger oil showed weaker inhibition, with zones ranging from 1 mm to 5 mm. Among the formulations, G<sub>40</sub> (40 µL ginger essential oil) demonstrated the highest activity, particularly against *S. typhimurium* (D = 4.00 ± 1.00 mm) and *E. coli* (D = 4.33 ± 0.58 mm).

Previous studies by Wang et al. (2020) reported that the volatile oils of ginger, including zingiberene, α-curcumene, and 6 – gingerol, could inhibit the expression of genes linked to bacterial energy metabolism, decreasing the metabolic activity of bacteria [32]. In this study, the inhibition zone of the candy extracts was lowest for *S. aureus*, with no significant difference among treatments. This can be attributed to the complex composition of essential oils and the oil extraction methods, leading to varying antimicrobial effects. Wang et al.'s study showed a much larger inhibition zone (17.1 mm) for ginger essential oil against *S. aureus*, likely due to the use of pure essential oil, whereas this study used candy extract containing at least 10 µL of essential oil in a 125 – gram batch of candy. Even though the candies were formulated with propolis and honey, the antimicrobial activity of these candy extracts against *Salmonella*, *S. aureus* and *E. coli* was still significantly weaker than streptomycin. These findings underscore the importance of optimizing concentration levels of those bioactive compounds to achieve the best antimicrobial effects.

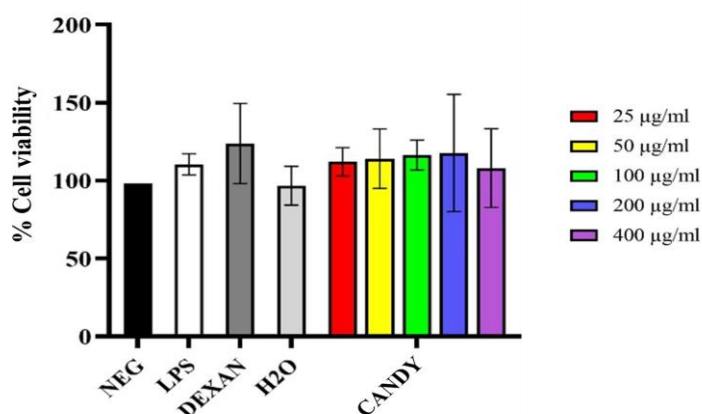
### 3.5. Anti-inflammatory effect of formulated candy

Based on the results of the sensory test, among the four samples, the candies containing 10 µL and 20 µL of ginger essential oil (GEO) received the highest scores. Higher concentrations of GEO, such as 30 µL and 40 µL, resulted in an unpleasant taste and a strong tangy flavor, leading to lower scores for these samples. Additionally, while the total polyphenol content of these two samples was not significantly different, the candy with 10 µL of GEO exhibited a higher inhibition zone against tested pathogenic bacteria. Consequently, this candy was selected for further testing to evaluate its anti-inflammatory properties and other parameters.

**Table 7.** Evaluating the inhibition of NO secretion in experimental samples

Samples	NO (%)
NEG	3.13 ± 0.79 <sup>c</sup>
LPS	14.99 ± 1.06 <sup>a</sup>
DEXAN	6.78 ± 0.25 <sup>b</sup>
H <sub>2</sub> O	15.37 ± 5.03 <sup>a</sup>
CANDY-25	17.06 ± 0.79 <sup>a</sup>
CANDY-50	15.51 ± 2.89 <sup>a</sup>
CANDY-100	14.44 ± 1.35 <sup>a</sup>
CANDY-200	17.90 ± 6.65 <sup>a</sup>
CANDY-400	11.95 ± 3.55 <sup>a</sup>

Values (Mean ± Standard deviation) with different superscripts in the same column are significantly different (one-way ANOVA and Tukey test,  $P < 0.05$ ). In which NEG is negative control, LPS is Lipopolysaccharide, DEXAN is Dexamethasone, CANDY is propolis-enriched ginger honey candy at different concentration (µg/mL).



**Figure 2.** Evaluation of the cytotoxicity of honey candy supplemented with propolis and ginger essential oil on RAW264.7 cells after inflammation induction.

The study indicates that formulated candy does not strongly inhibit nitric oxide (NO) secretion in LPS-induced RAW264.7 cells, with NO levels only slightly decreasing from 17,06 % to 11,95% as the candy extract concentration increased from 25 µg/mL to 400 µg/mL. However, this reduction remains significantly higher than the dexamethasone - treated control group (Table 7), suggesting that the anti-inflammatory potential of the formulated candy through NO inhibition is limited. A key finding is that formulated candy does not exhibit cytotoxicity, as cell viability remained above 96% at all tested concentrations, demonstrating the product's high safety profile (Figure 2).

### 3.6. The physicochemical analysis of the ginger honey candy supplements with propolis

#### 3.6.1. Total soluble solid (TSS)

The total soluble solid content of the candy samples G<sub>10</sub> was 87%. The Brix values of the hard candy samples were within the suitable range for hard candies. The ideal Brix values for hard candies are typically between 70 and 80°Brix [33], [34]. The addition of GEO does not result in variation of Brix values. The TSS in the current study's samples might affect the sweetness and overall texture of the candies, potentially influencing consumer acceptance.

#### 3.6.2. Moisture content

The moisture content of the candy samples G<sub>10</sub> was found to be  $2.9 \pm 1.84\%$ , which falls within the expected range for hard candies (2-3%) (TCVN 5908:2009 standard). This moisture level is crucial for maintaining the stability and glassy state of the candies, preventing them from becoming sticky or prone to microbial growth. The consistency in moisture content with published values suggests that the candies are likely to have a similar shelf life and storage stability as other hard candies [35], [36].

#### 3.6.3. Hardness

The texture of hard candies is another crucial quality parameter. The moisture content and hardness of candies determined the hard candy's final texture. Regarding hardness, the candy samples exhibited a value of  $531.11 \pm 5.52$  g. The higher hardness of the current samples may impact their chewability and consumer preference, as excessively hard candies might be less desirable. It is essential to balance the hardness to ensure a pleasant eating experience while maintaining the structural integrity of the candies.

#### 3.6.4. Colorimetry

In terms of color, the sample containing 10 µL of GEO (G<sub>10</sub>) exhibited the following values: L =  $35.85 \pm 0.07$ , a =  $7.62 \pm 0.26$ , and b =  $16.71 \pm 0.22$ . These values indicate a light brown color with moderate redness and yellowness. Previous studies on honey candies have shown a wide range of color values depending on the type of honey and processing methods [37]. Therefore, the color of candy can be affected by the type of honey used. Beside the effect of honey's color, the light brown color of this candy aligns with findings from Kaushal et al. (2020), who linked ginger's polyphenols to brownish

hues [38]. The redness (a value) is consistent with Otmani et al. (2021), who found that phenolic-protein interactions during heating enhance red tones [39]. In terms of yellowness (b value), G<sub>10</sub> exhibited the strongest yellow hue (16.71 ± 0.22), likely due to oxidation reducing gingerol pigmentation [24]. These results underscore that optimizing ginger and propolis ratios is crucial for enhancing sensory appeal and consumer acceptance, as color significantly impacts marketability.

#### 4. Conclusions

The study aimed to develop a hard candy utilizing Vietnam's abundant honey supply, combined with ginger essential oil and propolis. Sensory evaluation indicated that 10% honey formulation was the most preferred. Among the propolis concentrations (0.5 – 2%), the candy containing 0.5% propolis was the most favored. The candy with 10 µL ginger essential oil was chosen as the best formula in this study achieving highest sensory score while still exhibiting some potential antioxidant and antimicrobial activities. The results demonstrated that the combination of propolis and ginger enhances the honey candy's antioxidant and increases the total polyphenol content compared to commercial hard candies. Therefore, the honey ginger candy supplemented with propolis has a distinctive color and flavor, meeting consumer preferences while retaining the inherent antioxidant properties of natural ingredients like propolis and ginger.

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#### Conflict of Interest

All authors declare no conflict of interest.

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