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Biodegradable films incorporating Malaysian stingless bee propolis: Development, characterization, and potential for food packaging

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ABSTRACT

Three different Malaysian stingless bee propolis samples were examined using the ethanolic extraction method for total flavonoid (TFC) and phenolic content (TPC), antioxidant, and antibacterial activities. Additionally, the biodegradable films were developed and characterized, this study also aims to enhance the functional qualities for possible use in active food packaging by combining corn starch (CS) with propolis extract (PE). The propolis samples were extracted with 70 % ethanol and analysed through a UV–VIS spectrophotometer for determination of antioxidant, TPC, and TFC. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as scavenging test for free radicals, while Folin-Ciocalteu and aluminium chloride ($AlCl_3$) were used to measure total flavonoid and phenolic contents. The data presented in this study showed significant differences in TPC and TFC identified in each sample ranged from 31.95 to 59.48 mg/mL GAE and 53.88 to 59.49 mg/mL RE, respectively. The findings also showed significant differences in the antibacterial activities of Malaysian stingless bee propolis, especially against Gram-positive bacterial strains. In comparison to the control treatments, the ethanolic propolis extract treatments also improved the film's physiochemical and antibacterial qualities. The incorporation of PE into CS resulted in decreased moisture content of the films from 17.20 % to 14.39 %, whereas the solubility significantly decreased from 17.44 % to 12.14 %. The weight of CS and PE film lowered significantly after 14 days and the weight loss percentage also demonstrated that bioplastic degradation occurred. The propolis extract was able to prevent the growth of foodborne bacteria since the present data revealed that the microbial count was significantly lower than control groups by displaying an acceptable limit of aerobic plate count for red meat products, which is lower than 6 log CFU/g. Propolis from Malaysian stingless bees may offer a viable substitute for synthetic additives in biodegradable food packaging films. Its antimicrobial and antioxidant properties support its application for sustainable food preservation.

1. Introduction

The distribution of stingless bee species is found to be more diverse in the environment due to its eusocial behavior. They are highly apt to various adaptations in the environment, which make them a good plant pollinator in the agriculture sector (Hrncir et al., 2016). Stingless bees compensate the absence of defense organ by producing "bee glue" or propolis from other predators as a way of protecting themselves (Anjum et al., 2019). According to Sambou et al. (2020), propolis is a resinous substance produced by mixing resin (sticky liquid) collected from leaves, flower buds, stems, and bark cracks of numerous vegetations with their saliva and beeswax.

Propolis is widely recognised for its beneficial therapeutic effects, which include antibacterial, antioxidant, antifungal, anti-obesity, anti-cancer, and many more (Machado et al., 2016; Kia et al., 2018; Santos et al., 2020; Vargas- Sanchez et al., 2020). A study by Ismail et al. (2017) found that ethanolic propolis extract with chitosan can develop a low-cost active food packaging because of its antibacterial effects, which is crucial for food preservation. The main components of propolis are resins, waxes, pollen, essential oils, and minerals, such as amino acids,

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lipids, polyphenols, and vitamins. (Rufatto et al., 2017; Kocot et al., 2018). The effectiveness of biological activities exhibited by propolis varied in different countries due to varying geographical areas, seasonality, vegetation sources, bee species, and collecting seasons (Nordin et al., 2018; Nascimento et al., 2019).

The addition of propolis in food production can also be an alternative to chemical food preservatives due to its high concentration of bioactive compounds in propolis; particularly the phenolic and flavonoid compounds (Pasupuleti et al., 2017; Marly et al., 2018). Besides, the use of propolis in food has also been recognized by Generally Recognized as Safe (GRAS) as a new natural preservative agent in food productions (Tzima et al., 2015; Saricoban & Yerlikaya, 2016). According to Yaacob et al. (2018), natural products such as propolis can limit the application of synthetic preservatives that might affect the health of consumers in the long term. Moreover, several studies have also reported the richness of properties in propolis that can help to enhance the quality and durability of food during storage (Pobiega et al., 2019b).

It is possible to make biodegradable and edible packaging materials for meat products from polysaccharides obtained from fruits and vegetables. Biodegradable films used in preserving meat products represent a novel technique, as the antimicrobials and antioxidants can be added directly to the film-casting solution or sprayed directly on the film surface. Bio-based packaging films with natural additives help to improve the films' properties and functionalities as well as the meat product's quality. According to Suriyatem et al. (2018), adding propolis to rice starch biodegradable films may improve their antibacterial and antioxidant qualities. Besides, findings from Nauman et al. (2022) demonstrated a promising food bio-packaging with great thermal stability, mechanical properties, antioxidant, and antimicrobial activities when chitosan-based film was enriched with propolis extracts.

Therefore, this study was carried out to investigate the total phenolic and flavonoid compounds as well as the antioxidant activities of propolis from three Malaysian stingless bee species (*Homotrigona fimbriata, Tetrigona apicalis, and Tetrigona binghami*). The Malaysian stingless bee propolis used in this study were chosen due to their nesting site located in the forest, which is generally less exposed to pollutants and pesticides, besides having diverse plant sources, which results in various bioactive compounds in the propolis. The analysis was carried out to identify the chemical components in stingless bee propolis extracts studied since reports on the chemical contents of these Malaysian meliponine propolis are very limited. Additionally, the development of food bio-packaging supplemented with propolis extract and food preservation testing were also conducted. The physiochemical and antibacterial characteristics of the films, as well as the microbiological testing of beef samples, were demonstrated since propolis may potentially be a natural additive for utilisation in the food preservation sector since it had been proven to be much safer, environmentally friendly, and biocompatible compared to the synthetic additives.

2. Materials and methods

2.1. Sample collection

A fresh collection of propolis samples was obtained in June 2020 from *H. fimbriata, T. apicalis, and T. binghami* species at the Malaysia Genome Institute (N 2° 54′ 16.8732″ E 101° 46′ 5.61″). After that, each sample was kept at −20 °C in the dark to avoid photodegradation until further study was done (Mohamed et al., 2020).

2.2. Preparation of ethanolic propolis extract

Some modifications on the method for extracting samples was prepared in accordance with Kia et al. (2018) Forty grams of the raw sample was processed into powder and 100 mL of 70 % ethanol was added. After 5 min of heating at 70 °C, the mixture was allowed to cool at room temperature overnight under dark condition. After 24 h, Whatman No 1 was used to filter the mixture and evaporated using vacuum rotary evaporator (EYELA Digital Water Bath SB-1000) at 40 °C for 30 min. Next, the dry extract was scraped from the round bottom flask surface before keeping at −20 °C in the dark.

2.3. Determination of total phenolic and flavonoid compounds

The total phenolic contents were estimated using the Folin-Ciocalteu colorimetric method as outlined in the study by Johari and Heng (2019), with some minor adjustments. To create the Folin-Ciocalteu stock solution (1:1), 0.2 mL of the Folin-Ciocalteu reagent and 0.6 mL of 95 % ethanol were mixed together. Then, 0.2 mL of propolis extract was added to the freshly prepared stock solution. After a 5-minute incubation period, 1 mL of an 8 % sodium carbonate solution and 3 mL of 95 % ethanol were added to reach the final volume. The solution was kept in darkness for 50 min, then the absorbance was measured at 725 nm using a UV–VIS spectrophotometer (DU 730 Beckman Coulter). A standard calibration curve was developed using gallic acid.

The total flavonoid compound was analysed using the aluminum chloride ($AlCl_3$) colorimetric technique, as per the method by Hossain et al. (2019) with some modifications. A standard calibration curve was established using rutin (mg/mL RE) over a range of 25–250 μg/mL. For each standard and sample solution (0.2 mL), 0.5 mL of potassium acetate (1M) and 0.5 mL of aluminum chloride (10 %) were added. The solutions were left at room temperature for 30 min without exposure to light. The absorbance was measured at 510 nm using a UV–VIS spectrophotometer (DU 730 Beckman Coulter).

2.4. 2,2-Diphenyl- 1-picrylhydrazyl (DPPH) free radical-scavenging assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging (Alfa Aesar, Thermo Fisher Scientific, USA) technique, as previously reported by Frezzini et al. (2019), was slightly modified in order to determine the antioxidant activity of propolis extracts. Depending on the compound's electron transport, this stable free radical will change from violet to yellow or colourless. Methanol and ascorbic acid (Sigma Aldrich, USA) were used as blanks.

DPPH reagent (4 mg) was combined with 100 mL of methanolic solution (0.004 %) to create a fresh 1.0 mM DPPH stock solution. Subsequently, 3.0 mL of DPPH stock solution was added to a test tube with 1.0 mL of each propolis extract. The mixture was shaken gently to mix them and placed at ambient temperature under dark condition for 30 min. The absorbance measurements were fixed at 517 nm using UV–VIS Spectrophotometer (DU 730 Beckman Coulter). All samples were analysed in triplicate.

The percentage of DPPH scavenging activity was calculated using equation shown below and the IC_{50} inhibition value was determined through equation based on the concentrations of propolis extracts and its respective DPPH scavenging activity percentages. The control sample's absorbance is represented by A_{blank}, while the sample's absorbance using DPPH stock solution is represented by A_{sample}. In the end, the DPPH activity result was reported as IC_{50} values, which indicated the sample concentration required to block 50 % of the DPPH free radical. Low antioxidant capability is indicated by a high IC_{50} value.

$$\text{DPPH Scavenging Activity } (\%) = \frac{(A\ blank\ - A\ sample)}{(A\ blank)} \times 100\%$$

2.5. Antibacterial activity of propolis extract

The antibacterial activity was investigated by conducting the disk-diffusion method in order to test the effectiveness of the antibacterial properties in the sample as documented by Del et al. (2018) with minor modification. On nutrient agar plates, the bacterial strains (*Staphylococcus aureus* ATCC 35,556*, Bacillus subtilis* ATCC 6051*, Escherichia coli*

ATCC 25,922, and *Salmonella typhi* ATCC 14,028) were cultivated and incubated for 24 h at 37 °C. After that, they were refrigerated and stored at 4 °C. Next, using a sterile cotton swab, the bacterial strains were evenly distributed on Mueller-Hinton agar plates. Then, the 6 mm filter paper discs loaded with positive control (cefotaxime, antibiotic), negative control (sterile distilled water), and pure sample (propolis extract) were placed on the agar plate's surface. Finally, the inhibition zone around the discs was calculated after 24 h of incubation at 37 °C. The final value of inhibition zones, including the diameter of the disc on the agar plates must be > 6 mm to be considered active against bacteria. The tests were performed in triplicates.

2.6. Preparation of CS film incorporated with PE

The solution casting method was done in Universiti Putra Malaysia's Laboratory of Environmental Studies to create the CS-PE films. To create a homogeneous solution, 30 % (w/w, starch basis) glycerol was first added to a beaker holding distilled water (180 mL). The beaker was then heated at 85 °C for 20 min using a water bath. Glycerol was used as a plasticizer to reduce the molecular bonding power of starch so that the flexibility of the film is increased. Next, 10 g of corn powder and propolis extract with 0 % and 2 % (*w/w*, starch basis) were introduced into the prepared solution. For 20 min, the solution was heated to the same temperature in a water bath and the slurry was then left to cool at room temperature before being casted on petri dish.

To ensure consistency in film thickness, 50 g weights were placed on the casting dishes. Next, all the casting dishes were placed into the oven at 45 °C for 18 h until completely dried. The casting dishes were then kept at room temperature for a day before all films were removed from the Petri dishes and stored in a humidity chamber set to 25±2 °C at 50 % ±5 % relative humidity for one week prior to characterization (Tarique et al., 2021).

2.7. Physiochemical characteristics CS film incorporated with PE

2.7.1. Film thickness

A digital micrometre (Mitutoyo Corp., Japan) was used to measure the thickness of each film sample with an accuracy of 0.001 mm five times at different locations and the mean thickness of the film was then determined (Marichelvam et al., 2019).

2.7.2. Moisture content

The amount of moisture that a dry film absorbs from the environment until the moisture content of the film and the surrounding air equalise is called the moisture content of the film. The films were divided into 2 cm by 2 cm square pieces and then weighed to determine the moisture content. The weight of the wet sheets at the beginning was referred to as Wm. The films were then held in a vacuum oven at 105 °C until they reached a uniform weight. The term "Wd" refers to the dry weight of the films (Marichelvam et al., 2019). The following formula was used to determine the moisture content:

$$\textit{Moisture content}\ \% = \frac{(Wm - Wd)}{Wm} \times 100\%$$

2.7.3. Film solubility

The amount of dry matter in the film that dissolves in water is used to determine the soluble content of the films. The solubility of the films was evaluated using modified methods from previously published research (Adilah et al., 2018). The films were cut into 2 cm by 2 cm square pieces and thoroughly dehydrated before being stored in a vacuum desiccator. The films were periodically weighed until they reached a constant weight, indicating complete drying; this weight was referred to as the initial dry weight. Subsequently, the films were continuously stirred at 25 °C for twenty-four hours after being immersed in 50 mL of deionized water in a beaker. Once removed from the beakers, the films were dried to a constant weight at 105 °C. This weight was referred to as the final dry weight. The following formula was applied to calculate the solubility percentage.

$$\text{Water solubility}\ \% = \frac{(\textit{Initial Dry Weight} - \textit{Final Dry Weight})}{\textit{Initial Dry Weight}} \times 100$$

2.7.4. Biodegradability characteristics of CS film incorporated with PE

A biodegradability test was conducted to determine the biodegradability of film in a given or intended-use environment. According to Fauziyah et al. (2021), to determine the biodegradability (according to ISO 14,855:1999), 4 cm×4 cm square pieces of films were weighed. This initial weight of the film was denoted as W_1. The films were then kept in a UV polybag (8 × 8 inches) containing 500 g of soil (nitrogenous bacteria) and buried at a depth of 10 cm for 14 days under room conditions. The films were weighed after 14 days and denoted as W_2. The biodegradability test was calculated using the following equation:

$$\textit{Weight loss}\% = \frac{(W1 - W2)}{W1} \times 100\%$$

2.8. Antibacterial activity of CS-PE film

The effectiveness of the film's antibacterial properties was assessed using the disc-diffusion method following the protocol outlined by Gheibi and Samiee-Rad (2020) with minor adjustments. Nutrient agar plates were used to culture standard bacterial strains (*S. aureus* ATCC 35, 556, *B. subtilis* ATCC 6051, *E. coli* ATCC 25,922, and *S. typhi* ATCC 14, 028) at 37 °C for 24 h, after which they were stored at 4 °C. The bacterial strains were then evenly spread onto Mueller-Hinton agar plates using a sterile cotton swab. 6 mm filter paper discs containing the positive control (cefotaxime, an antibiotic), negative control (distilled water), and film samples (corn starch and propolis extract) were positioned on the agar plates. After 24 h of incubation at 37 °C, the inhibition zone around the discs was measured. To be considered active against bacteria, the combined diameter of the disc and the inhibitory zones on the agar plates had to exceed 6 mm. The tests were conducted in triplicate.

2.9. Food spoilage testing

2.9.1. Sample collection

A total of nine beef samples (5 g each) were collected from a selected butcher shop early in the morning, between 8:00 and 9.00am, at Pasar Awam Taman Seri Serdang, Seri Kembangan, Selangor (N 2° 33′ 58.896″ E 102° 43′ 26.4″), using sterile polythene plastic bags. The samples were then transported to the Faculty of Environmental Studies, Universiti Putra Malaysia laboratory using an icebox before being stored for further analysis. (Atlabachew & Mamo, 2021).

2.9.2. Sample storage

The samples were cut and stored after being wrapped to avoid cross-contamination. Each sample was weighed before being stored at 4 °C for 14 days. The samples were then tested on day 14 for weight and microbiological testing (Diyantoro & Wardhana, 2019; Nauman et al., 2022). The meat samples were divided into 4 groups as follows:

A: Beef without any wrapping packaging.

B: Beef with polyethylene plastic.

C: Beef with corn starch-based film incorporated with propolis extract film.

D: Beef with corn starch film.

2.9.3. Weight loss

According to Martinek et al. (2022), all samples were thawed under refrigeration before further analysis the next day. The initial weight of the sample was denoted as W_1 and the samples were weighed again after 14 days after stored cold storage and denoted as W_2. The weight loss was

calculated using the following equation:

$$Weight\ loss\% = \frac{(W1 - W2)}{W1} \times 100\%$$

2.9.4. Determination of pH in beef samples

The samples were first defrosted in the refrigerator before further analysis the following day. A digital pH meter was used to measure the pH of the samples by inserting the probe into the meat for 1 min, and the reading for each sample was recorded. Prior to taking the measurement, the pH meter was calibrated using a standard buffer solution (neutral) and rinsed with distilled water after each measurement. Initially, the pH was measured before the samples were stored at 4 °C, and finally, the same test was conducted after 14 days of storage (Gebrehiwot et al., 2018).

2.9.5. Sampling procedure

The samples were initially thawed under refrigeration before further analysis the next day. The weight loss of the samples was monitored using digital balance after 14 days in the refrigerator. Next, the samples were placed into a sterile homogenizer blender with 50 mL of 1 % Buffered Peptone Water (BPW) for 5 mins and then placed into a sterile beaker. The homogenized samples were left at room temperature at 20 ± 2 °C for 8 – 10 mins before being put into serial dilution (Evangelista-Barreto et al., 2022).

2.9.6. Microbiological testing

According to Nauman et al. (2022), Total Plate Count (TPC) was used to assess the results of the microbiological testing of beef samples. For the purpose of serial dilution, five sterile test tubes were labelled as 10^{-1} to 10^{-5}. In the first test tube, 1 mL of diluted meat sample and 9 mL of BPW were then thoroughly mixed and marked as 10^{-1}. Next, a 1 mL solution was extracted from the first test tube and moved to the 10^{-2} test tube. Until a 10−5 dilution was obtained, the process was repeated. Subsequently, 100 µL of meat samples from every dilution were injected into Plate Count Agar (PCA) plates, which were subsequently incubated for a whole day at 37 °C.

2.10. Statistical analysis

Mean ± standard error of mean was used to express the data. ANOVA, a one-way analysis of variance, was utilised for all bioactive compound using the Statistical Package for Social Sciences (IBM SPSS Statistics Software, 22.0 version). The values obtained from this experiment, $p \leq 0.05$ were considered statistically significant. All tests were carried out in triplicate.

3. Results and discussion

3.1. TPC and TFC of propolis ethanolic extracts

Tables 1 and 2 list the concentrations of biochemical compounds observed in the ethanolic extracts of stingless bee propolis, including the flavonoid and phenolic contents. Analyses of all the samples reveal no significant differences in any of the compounds investigated. It can be seen that *H. fimbriata* had the highest concentrations of compounds for all propolis samples tested, while *T. binghami* were the lowest for all the extracts.

Table 1
Total phenolic contents (TPC) in different propolis ethanolic extracts.

Propolis	TPC (mg/mL GAE)
H. fimbriata	59.48 ± 0.05*
T. apicalis	42.15 ± 0.03*
T. binghami	31.95 ± 0.03*

* Concentration is significantly difference at $p \leq 0.05$.

Table 2
Total flavonoid contents (TFC) in different propolis ethanolic extracts.

Propolis	TFC (mg/mL RE)
H. fimbriata	59.49 ± 0.36*
T. apicalis	54.75 ± 0.07*
T. binghami	53.88 ± 0.04*

* Concentration is significantly difference at $p \leq 0.05$.

In addition, the extract of *H. fimbriata* had the highest total phenolic content (59.48 mg/mL, followed by the *T. apicalis* extract of (42.15 mg/mL) and the extract of *T. binghami* (31.95 mg/mL) had the lowest phenolic content. Another study of propolis extracts from Malaysian stingless bees showed that the extract of *H. fimbriata* had the highest concentration of phenols (16.2 mg/mL), followed by extracts from *T. apicalis* (13.9 mg/mL) and *T. binghami* (5.7 mg/mL) (Awang et al., 2018). In addition, propolis extracted from stingless bees from Indonesia showed a total phenolic content between 10 and 28.65 mg/mL (Fikri et al., 2019). Phenols are crucial for propolis to exhibit strong biological effects such as antioxidant activity. Besides, they also benefit human health by rejuvenating cells, exhibit antimicrobial, anticancer as well as anti-ulcer activities, among others (Badiazaman et al., 2019).

Next, the highest total flavonoid content was observed in the propolis extract of *H. fimbriata* at 59.49 mg/mL, while the extract of *T. apicalis* yielded 54.75 mg/mL and *T. binghami* contained the least amount of flavonoids at 53.88 mg/mL. A study conducted by Fernandes et al. (2015) on propolis from Malaysian stingless bees, including *T. apicalis*, yielded high amounts of flavonoids, resulting in high antioxidant activity of the extracts. Furthermore, propolis extracts from *A. mellifera* and Mexican *Melipona beecheii* produced flavonoids with 7.68 and 17.23 mg g^{-1}, respectively (Rufatto et al., 2017). The composition (flavonoids and others) of propolis mainly depends on its botanical origin (resin sources) and the type of stingless bee species (Rosli et al., 2016; Awang et al., 2018)

Ethanol as a solvent is suitable to be used when extracting polyphenolic compounds in the propolis, while water is preferred to obtain compounds such as phenolic acids that are more water soluble (Pobiega et al., 2019a). Moreover, ethanolic solvent contains lipophilic materials, which helps fats and non-polar solvent in propolis to pass through the cell membrane easily compared to non-ethanolic extract such as water and oil. Therefore, it is important for the propolis extracts to contain high polyphenolic components that can yield effective biological activities, especially when being utilized in food and health industries.

Biodegradable packaging material incorporated with propolis extract can also protects the quality of food products, mainly perishable food such as meats, poultry meats, fruits, and vegetables. Propolis shows the ability to be used as an effective natural additive in film due to it containing a variety of polyphenolic compounds (phenolic acids, flavonoid, hydrocarbons, and terpenoids), which mostly contributes to the biological activities displayed by the propolis samples (Siripatrawan & Vitchayakitti, 2016).

3.2. Total antioxidant activity and correlation between antioxidant activity with TPC and TFC of propolis ethanolic extracts

Table 3 shows that *H. fimbriata* recorded the lowest IC_{50} value (5.06 mg/mL) compared to *T. binghami* (11.72 mg/mL) and *T. apicalis* (7.17 mg/mL) with a good correlation coefficient, $r^2 = 0.9806$. Different amounts of DPPH and IC_{50} values observed in different propolis extracts are due to factors such as floral preferences, bee species as well as plants pollinated by the bees (Awang et al., 2018). Suriyatem et al. (2018) also proved that the ethanolic propolis extracts had better antioxidant and antibacterial characteristics, which are great for food sector utilisation as active food bio-packaging. Campos et al. (2015) also concluded that

Table 3
The percentage of DPPH scavenging activity and IC_{50} value of propolis ethanolic extracts.

Propolis	Concentration of Sample (mg/mL)	DPPH Scavenging Activity (%)	IC_{50} (mg/mL)
H. fimbriata	1	3.19±0.05	
	2	18.00±0.08	5.06±0.02
	3	29.38±0.18	
	4	34.85±0.08	
	5	50.34±0.18	
T. apicalis	1	2.05±0.06	
	2	14.81±0.15	7.17±0.04
	3	24.83±0.04	
	4	28.02±0.12	
	5	31.21±0.14	
T. binghami	1	24.15±0.22	
	2	25.28±0.09	11.72
	3	28.47±0.08	±0.03
	4	31.21±0.03	
	5	33.50±0.07	
Ascorbic acid	1	19.17±0.05	
	2	24.25±0.05	5.08±0.01
	3	37.19±0.04	
	4	42.13±0.02	
	5	48.22±0.04	
	Blank	0.00±0.00	

propolis extracted from *Trigona* spp. contain polyphenols that can undoubtedly be advantageous in the food and health sectors.

Besides, the correlations of TFC and antioxidant activities (r = 0.963), as well as TPC and antioxidant activities (r = 0.896) of the extracts show that they strongly correlate with each other, as shown in Table 4, indicating that the higher the values of phenolic and flavonoid contents determined in the extracts, the higher the antioxidant activities that can be obtained from the extracts. However, there is no correlation between the TFC and TPC found in these propolis extracts with r = 0.138. Similarly, Pobiega et al. (2019b) also showed an overall strong positive correlation between the total phenolics and flavonoids with their antioxidant activities in the propolis extracts tested.

According to Awang et al. (2018), Malaysian stingless bees, including *T. apicalis, H. fimbriata, T. binghami* and *Heterotrigona itama* contain flavonoids and phenolics compounds that can be useful in producing biologically active substances and producing strong antioxidant activities when studied. Other than that, Salim et al. (2018) had also successfully proven that the TPC, TFC and antioxidant activities derived from the ethanolic propolis extract of the Malaysian *Geniotrigona thoracica* in some cases are even better than those obtained from other countries.

Furthermore, a study done on Bruneian *H. itama, T. binghami* and *G. thoracica* propolis extracts also showed significant results in their biological activities, which could potentially be due to their high phenolic and flavonoid contents (Abdullah et al., 2020). Moreover, research carried out on Mexican propolis collected during the summer yielded extracts with higher total flavonoid and phenolic contents than other seasons, which contributed greatly to their antimicrobial and antioxidant activities, thus proving that there are various factors that could influence the quality of the propolis extracts (Vargas- Sanchez et al., 2020).

Table 4
Pearson's correlation coefficient between antioxidant activity with TFC and TPC in propolis ethanolic extracts.

Assay	TFC	TPC	DPPH
TFC	1	0.138	0.963*
TPC	0.138	1	0.896*
DPPH	0.963*	0.896*	1

* Correlation is significant at $p \leq 0.05$.

3.3. Antibacterial activity of propolis ethanolic extracts

Table 5 records diameters of inhibition zones exhibited by the propolis ethanolic extracts studied when tested against Gram-positive (*B. subtilis* and *S. aureus*) and negative (*S. typhi* and *E. coli*) bacteria. The propolis extracts were observed to be highly susceptible to both Gram-positive bacteria. However, no inhibition zone was formed when tested against Gram-negative bacteria. There were significant differences detected in the antibacterial activities of all propolis samples since $p < 0.05$.

This finding is also corroborated by Ghasemi et al. (2017) which noted that propolis extracts documented in previous studies were able to exhibit antibacterial activity against various Gram-positive strains but has very limited action when tested against Gram-negative bacteria. Moreover, Torres et al. (2018) had also reported Gram-positive bacteria, especially *S. aureus*, to be more susceptible than Gram-negative strains such as *E. coli* when tested against Brazilian *Melipona quadrifasciata* propolis ethanolic extracts. It were also stated that propolis extracts from different places had reported different results due to factors such as solvents used as well as botanical origins (Yusop et al., 2019). Besides, propolis extracts had also been noted to contain polyphenolic and flavonoid compounds that exhibit antimicrobial activities that were useful pharmacologically (Gheibi & Samiee-Rad, 2020).

Solvents used when extracting propolis are also important in determining the chemical compounds and biological activities displayed by the propolis. Propolis ethanolic extracts are commonly used as extraction solvents because they are organic in nature and suitable to be used when extracting polyphenolic compounds in propolis such as flavonoids that are highly soluble in alcoholic solutions (Rocha et al., 2013; Pobiega et al., 2019a). Furthermore, important compounds in propolis such as terpenoids, phenolic acids, and aromatic acids are mostly lipophilic, which are also solvable in ethanol and methanol (Kubiliene et al., 2018).

3.4. Physiochemical properties of CS film incorporated with PE

The CS film incorporated with PE was tested using ethanolic PE since the present study showed that ethanolic extracts were able to yield higher total polyphenolic compounds and demonstrated better biological activities. *H. fimbriata* propolis sample was chosen as an additive for the development of biodegradable packaging since studies conducted showed it was able to exhibit stronger antioxidant and antibacterial effects compared to *T. apicalis* and *T. binghami*.

The thickness, moisture content, and solubility of biopolymer-based films play crucial roles in food packaging applications, since they can either positively or negatively affect the quality of stored food over time. Film thickness is a key factor, as it influences the mechanical strength, moisture content, solubility, biodegradability, and oxygen permeability of the films. Table 6 illustrates a slight variance in the thickness of propolis-corn starch films with a 2 % concentration of propolis extract in each film.

Suriyatem et al. (2018) reported similar results, stating that the low

Table 5
Zones of inhibition diameters (mm) of propolis ethanolic extracts against Gram-positive and negative bacteria.

Bacteria / Propolis	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
H. fimbriata	14.0 ± 2.0+	13.0 ± 1.0+	0.0 ± 0.0	0.0 ± 0.0
T. apicalis	16.5 ± 1.0	17.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
T. binghami	17.5 ± 1.0*	15.0 ± 1.0*	0.0 ± 0.0	0.0 ± 0.0
Corn starch	18.0 ± 0.0	18.0 ± 0.0	18.0 ± 0.0	18.0 ± 0.0
Cefotaxime	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Distilled water	14.0 ± 2.0+	13.0 ± 1.0+	0.0 ± 0.0	0.0 ± 0.0

* Significantly different from *H. fimbriata* at $p < 0.05$.
+ Significantly different from *T. binghami* at $p < 0.05$
Note: Values expressed as mean ± standard deviations.

Table 6
The thickness, moisture content, and solubility of CS film incorporated with and without PE.

Propolis	Thickness (mm)	Moisture content (%)	Solubility (%)
H. fimbriata	0.11±0.01$^{\lozenge}$	14.39±1.41	12.14±1.74$^{\lozenge}$
T. apicalis	0.09±0.01$^{+, \lozenge}$	14.87±1.88	12.97±1.31$^{\lozenge}$
T. binghami	0.12±0.01$^{\#, \lozenge}$	17.85±1.30	14.73±1.66$^{\lozenge}$
Control	0.09±0.01$^{\#, +, *}$	17.20±1.17	17.44±1.01$^{\#, +, *}$

$^{\#}$ Significantly different from *T. apicalis* at $p < 0.05$.
$^{+}$ Significantly different from *T. binghami* at $p < 0.05$.
* Significantly different from *H. fimbriata* at $p < 0.05$.
$^{\lozenge}$ Significantly different from corn starch at $p < 0.05$
Results were expressed as mean±standard deviation.

extract content in the film explained the lack of a substantial variation in the thickness of the films. Additionally, food bio-packaging made of propolis extract, carboxymethyl chitosan, and rice starch displayed consistent structure. This suggests great miscibility between the film matrix and thinner films.

Furthermore, the majority of plant extracts demonstrated an increase in film thickness, according to studies by Zhang et al. (2020) and Riaz et al. (2020). The chitosan-based film incorporated with plant extracts displayed a significant increase in their film thickness. They asserted that a higher solid content from plant extracts added to the film matrix disrupted the film matrix's ordered structure, and caused an increase in the film thickness. Due to the hydrophilic nature of polysaccharides, biodegradable packaging typically lacks moisture barriers. Therefore, biopolymers such as starch are commonly incorporated with hydrophilic plasticizers such as polyols (glycerol, sorbitol, and polyethylene glycol) in order to avoid brittle films production.

These results are also comparable to those reported by Yong and Liu (2021) that found the presence of polyphenol compounds in the propolis extract enhances the hydrophobic properties, resulting in a good moisture barrier within the film matrix. Similar results were obtained by De Carli et al. (2022), in which tightened polymer chain interactions that resulted in the films' higher water barrier properties caused by the interactions between the hydrophilic groups of the chitosan-based film and the polyphenolic compounds of the propolis extract with polar properties were noted.

A study conducted by Chisenga et al. (2020) reported that biopolymer films tend to have low moisture barrier because of the large number of hydrophilic groups and the greater hydrogen bond interactions that occur between water molecules and their functional groups (-OH). However, the hydrophilicity of biopolymer films showed low water affinity when treated with different amounts of propolis extract. These results are also comparable to those of Khoshnevisan et al. (2019) who reported that the presence of wax which has hydrophobic qualities, helps propolis to adhere to a variety of surfaces, reduce gas exchange of food with air, and regulate the rate of transpiration and respiration of food.

A study conducted by Pérez-Vergara et al. (2020) stated that the solubility of the native cassava starch-based films was 41.98 % which showed a higher percentage of solubility compared to this study. However, the solubility of the film decreased to 21.62 % after the addition of propolis to the film. Due to the high concentration of long-chain fatty alcohols and alkanes in propolis, the hydrophobic agent (beeswax) might lower its water permeability.

Similar findings were also observed by Ismail et al. (2017), which mentioned an increase in propolis extract volume in chitosan-based film resulted in low solubility of the film. The untreated chitosan-based film, 1.2 mL and 2.4 mL of propolis-chitosan film showed solubility percentages of 80 %, 57.17 %, and 50 %, respectively. Thus, the beeswax from propolis acts as a hydrophobic agent which may be entrapped within the chitosan-based film matrix, creating strong interaction with the film network by hydrogen bonding, subsequently reduced the water affinity and solubility of the film. Fig. 1 shows the images of bio-packaging incorporated with and without stingless bee propolis ethanolic extracts after drying in the oven for 24 h.

Hence, the incorporation of propolis extract into polysaccharide-based films improved the water barrier properties. Low moisture content and solubility percentage are desirable parameters when producing a good biodegradable packaging, since it can protect perishable food

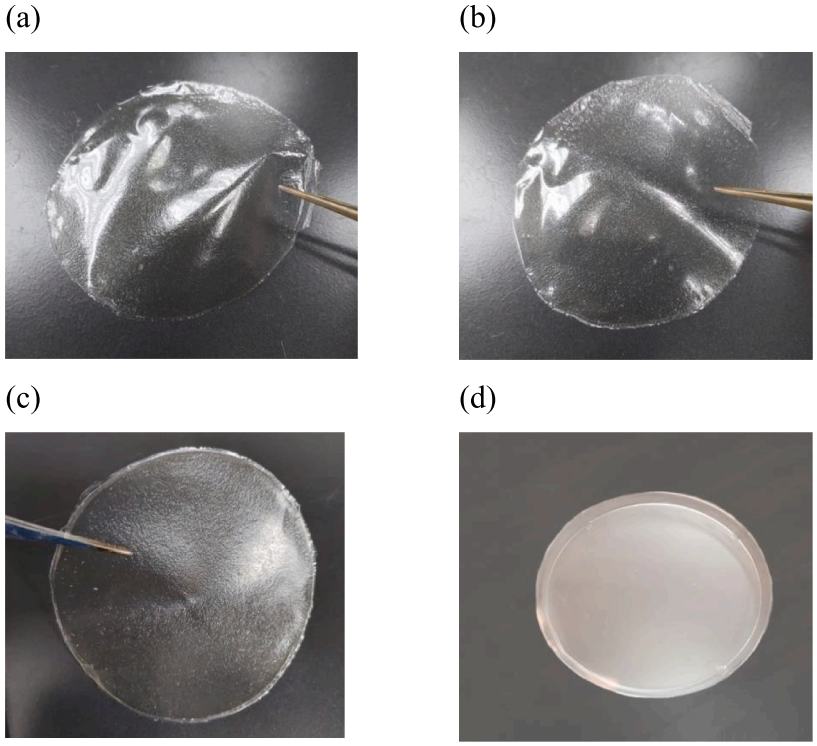

Fig. 1. The visual appearance of CS film incorporated with (a) *H. fimbriata* (2 %), (b) *T. apicalis* (2 %), (c) *T. binghami* (2 %), and (d) Control (CS without PE).

such as beef, poultry, vegetables, and fruits from deteriorating.

3.5. Biodegradability of CS film incorporated with PE

According to Nissa et al. (2019), the decomposing time of cassava starch film increased each day up to 29.89 % on day 10. Starch has acetal bonds which facilitates the degradation process and are easily digested by microbes. Moreover, starch granules were mainly composed of two main polymers namely amylose and amylopectin. The differences in structure and molecular weight between these polymers result in molecular and film-forming properties variation.

The presence of hydroxyl group (-OH) in a natural polymer was the main factor starch-based film can be easily degraded microbially (García-Guzmán et al., 2022). Rizwan and Jamal (2021) mentioned that the degradation process required an optimal condition such as humidity due to hydrophilic properties that commonly take place in moist environments. The secretion of amylase enzyme from various living organisms in soil such as bacteria, fungi, and worms facilitated the breakdown of polymer into monomer through hydrolysis. Hence, the insoluble starch materials can be converted into soluble products (maltose and glucose) which would be degraded by microorganisms.

The results shown in Table 7 are in agreement with those of Fauziyah et al. (2021) who stated that the addition of plasticizers such as glycerol, sorbitol, and polyethylene glycol during film-forming process will increase the water vapour permeability due to its hydrophilic properties.

The hydroxyl groups in glycerol will form hydrogen bonds with water, leading to reduced water resistance and enhanced water absorption properties. Temperature, oxygen levels, relative humidity, and microbial surroundings strongly affect the rate and mechanism of bioplastic material degradation (Laftah & Rahman, 2021). Consequently, it can be concluded that a higher concentration of glycerol will effectively promote a better bioplastic degradation process.

3.6. Antibacterial activity of CS film using PE

Table 8 showed the diameters of inhibition zones exhibited by the corn starch-based film incorporated with propolis ethanolic extracts studied when tested against Gram-positive (*S. aureus and B. subtilis*) and Gram-negative (*E. coli and S. typhi*) bacteria. The propolis extracts were observed to be susceptible towards both Gram-positive and negative bacteria, particularly *S. typhi*. There were significant differences detected in the antibacterial activities of all propolis samples since $p < 0.05$.

The differences in antibacterial properties between propolis extract and bio-packaging enriched with propolis extract might be due to several factors. Firstly, biopolymers used in bio-packaging development may have additional synergistic effects with propolis. Some polymers can enhance the diffusion or interaction of antibacterial compounds with microorganisms, thereby improving overall antimicrobial performance. Additionally, the matrix of the bio-packaging acts as a protective barrier, potentially preserving the antibacterial efficacy over time (Malm et al., 2021; El-Sakhawy et al., 2023).

This finding is also corroborated by Bertotto et al. (2022) which noted that starch-based film had no antimicrobial properties against all microorganisms. Moreover, Ardjoum et al. (2023) also reported that corn starch-based film without any propolis extract or essential oil additive did not show any antimicrobial action against foodborne bacteria. The addition of 10 % propolis extract and *Thymus vulgaris* essential oil showed potential antimicrobial activities against foodborne pathogenic bacteria that improved the bio-packaging functionality.

It has been stated that the chemical interactions of caffeic acid, quercetin, chrysin, pinobanksin, galangin, and other phenolic compounds released from the corn starch films enriched with propolis provide another potential explanation for the antibacterial activity of composite films (De Carli et al., 2022). Therefore, propolis extracts can be applied as a new and safe natural preservative for food packaging and other treatment applications due to the richness of polyphenolic compounds (El-Sakhawy et al., 2023).

Table 7
The weight loss percentage of CS film incorporated with PE using the Soil Burial Degradation Test after 14 days.

Propolis	Initial Weight, W_o (g)	Final Weight, W_f (g)	Weight Loss (%)
H. fimbriata	0.049±0.01	0.030±0.01◊	38.78±1.37#, ◊
T. apicalis	0.045±0.01	0.030±0.01◊	33.33±1.06*, +
T. binghami	0.048±0.01	0.031±0.01	35.42±1.29#, ◊
Control	0.053±0.01	0.023±0.01*, #	41.43±1.21*, +

* Significantly different from *H. fimbriata* at $p < 0.05$.
+ Significantly different from *T. binghami* at $p < 0.05$.
# Significantly different from *T. apicalis* at $p < 0.05$.
◊ Significantly different from corn starch at $p < 0.05$
Results were expressed as mean±standard deviation.

Table 8
Zone of inhibition diameters (mm) of corn starch-based film using PE against Gram-positive and negative bacteria tested.

Bacteria Propolis	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
H. fimbriata	12.0 ± 1.0*	10.5 ± 1.0*	0 ± 0.0	13.0 ± 1.0
T. apicalis	9.5 ± 1.0*	12.5 ± 1.0	0 ± 0.0	14.0 ± 1.0
T. binghami	0.0 ± 0.0	4.0 ± 1.0*	6.0 ± 1.0*	13.0 ± 1.0
Corn starch	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cefotaxime	23.5 ± 0.0	10.5 ± 0.0	14.0 ± 0.0	17.0 ± 0.0
Distilled water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

* Significant differences were detected between all groups at $p < 0.05$ using ANOVA.

3.7. Weight loss of beef

A study conducted by Leygonie and Hoffman (2020) reported that approximately 80 % of the water content in beef and poultry meat will be solidified into pure ice crystals, followed by a separation of dissolved solids. The speed of beef freezing will influence the size of the ice crystal formed, in which the faster the freezing speed the smaller the size of the ice crystals. All samples were stored under 4 °C following a slow freezing technique. Higher weight loss in Sample A might be due to the formation of large ice crystals in the beef. Large ice crystal will eventually disrupts the structure of the meat fibre resulting in higher weight loss of the meat (Deng et al., 2021).

Apart from those, the reduction in weight of food products during storage was also influenced by transpiration (loss of water from living tissues) and respiration (loss of carbohydrates). A research conducted by Kahramanoglu et al. (2020) reported that the inclusion of propolis extract with beeswax and terpenoid compounds improved the food's resistance to water vapor and made it more hydrophobic. Additionally, an increase in phenolic compounds in propolis helped to inhibit the movement of water and gases through the food's surface, resulting in excellent biodegradable properties for food products. These findings from Table 9 are similar to those of Pobiega et al. (2020), who found that

Table 9
The weight loss percentage of beef samples stored in cold storage after 14 days.

Sample	Initial Weight, W_1 (g)	Final Weight, W_2 (g)	Weight Loss (%)
A	5.00±0.01	4.45±0.35	11.0 %
B	5.00±0.01	4.77±0.12	4.6 %
C	5.00±0.02	4.82±0.03	3.6 %
D	5.00±0.01	4.55±0.21	9 %

No significant difference detected.
Results were expressed as mean±standard deviation.
A: Beef without any wrapping packaging.
B: Beef with polyethylene plastic.
C: Beef with corn starch-based film incorporated with propolis extract film.
D: Corn starch wrapping packaging.

a pullulan coating with ethanolic propolis extract enhanced the ability to resist water and delayed the ripening process of cherry tomatoes during 21 days of refrigeration.

The weight loss of uncoated cherry tomatoes were statistically significant higher compared to the coated tomatoes. Moreover, the addition of propolis extract did not impact the flavour and aroma of tomatoes, whereas the colour also appeared brighter which might improve consumer acceptability.

Besides, fresh blueberries with carboxymethyl cellulose (CMC) edible coatings + 1 % ethanolic propolis extract (EPE) showed lower percentage of weight loss 1.67 % than the uncoated blueberries (3.2 %) under 4 °C storage for 20 days. Other than that, CMC + 1 % EPE coated blueberries also demonstrated significant lower decay percentage (2.67 %) compared to untreated blueberries (17.69 %). Hence, treated blueberries showed better appearance and quality due to the presence of propolis which improved the shelf life of food and prevented moisture loss during storage (Tumbarski et al., 2022).

3.8. pH determination of beef

Table 10 showed the pH value of beef samples after being stored at 4 C for 14 days. The final pH value for Sample C (5.61) was significantly ($p \leq 0.05$) lower than that of Sample B (5.73), followed by Sample D (5.74) and Sample A (5.81). Significant differences were detected in the pH value of all beef samples at $p \leq 0.05$.

According to Jessira (2018), pH determination of beef has a significant role in determining its overall quality, taste, and freshness. The pH of fresh red meat should be in the range of 5.5 and 6.2. However, a decrease in pH (<5.3) may lead to undesirable qualities of meat due to poor preservation and improper storage. This study noted that beef wrapped with bio-packaging enriched with propolis extract showed an optimum pH level compared to other beef samples. Similar findings were also observed by Al-Azee et al. (2022), who reported that ground beef supplemented with 3 % of propolis showed an acceptable pH level of 5.84 after being stored at −18 °C for 30 days. The control group showed the lowest pH level of 5.32 after 10 days compared to other groups.

A study investigated by Vargas-Sánchez et al. (2019) also noted that the untreated beef and pork patties demonstrated a decrease in pH value, while sample groups enriched with 2 % ethanolic propolis extract showed higher pH values of 5.5 and 5.6, respectively after storage for 10 days in 2 °C. Furthermore, the authors also mentioned that by incorporating antioxidant properties from propolis, helps to improve the pH value of beef and pork patties. The studies conducted by Manessis et al. (2020) and Ismail et al. (2022) have established that antioxidant components, such as kaempferol, p-coumaric acid, caffeic acid, quercetin, propanoic acid, and volatile compounds from plant materials, which are also present in propolis, show potential as natural additives to enhance the quality of meat.

Table 10
pH of beef samples stored at 4 °C after 14 days.

Sample	Initial pH	Final pH
A	5.56±0.01	5.81±0.04$^{+\ \#}$
B	5.56±0.01	5.73±0.03$^{*\ \#}$
C	5.56±0.02	5.61±0.03$^{*\ +\ \lozenge}$
D	5.56±0.02	5.74±0.03$^{\#}$

* Significantly different from A at $p \leq 0.05$.
$^{+}$ Significantly different from B at $p \leq 0.05$.
$^{\#}$ Significantly different from C at $p \leq 0.05$.
$^{\lozenge}$ Significantly different from D at $p \leq 0.05$
Results were expressed as mean±standard deviation
A: Beef without any wrapping packaging
B: Beef with polyethylene plastic
C: Beef with corn starch-based film incorporated with propolis extract film
D: Corn starch wrapping packaging.

3.9. Total plate count of beef

Figs. 2 and 3 demonstrated the colonies formed and colony-forming unit (CFU/g) of beef samples stored at 4 °C for 14 days.

Based on previously published data, the maximum acceptability limit of aerobic plate count (APC) for red meat products are 6 log CFU/g, suggesting that the data obtained from this study were good meat quality and safe to be consumed (Shahbazi & Shavisi, 2018; Gedikoğlu, 2022). These findings showed the inhibitory properties of propolis against microbial growth on red meat products. Propolis exhibited great antibacterial activity due to the presence of chemical compounds, which include flavonoids, phenolics, and other bioactive compounds (Vargas-Sánchez et al., 2014).

These bioactive compounds were able to improve cell membrane permeability, resulting in the breakdown of bacterial cells (bacteriolysis). However, some researchers suggested that propolis was effective in reducing the number of Gram-positive bacteria compared to Gram-negative bacteria. The antibacterial properties of propolis in beef preservation involve multiple mechanisms, including disrupting bacterial membranes and inhibiting enzymes. The bioactive compounds, such as flavonoids and phenolic acids in propolis disrupt the microbial cell membrane containing proteins and nucleic acids which leads to cell death or impaired microbial function. Moreover, these compounds also inhibit enzymes responsible for cell wall synthesis, making them more susceptible to lysis (Almuhayawi, 2020; Bouchelaghem, 2022).

According to Jonaidi et al. (2018), they found that the outer membrane of Gram-negative bacteria limited the penetration and diffusion of hydrophobic compounds in propolis against bacteria. Adding ethanolic propolis extract (2 %) and wrapping raw beef patties in polyvinyl chloride (PVC) resulted in lower microbial counts over the storage period (4–7 log CFU/g) compared to untreated samples. Moreover, lipid oxidation and the red colour of beef patties were inhibited and preserved after being stored for 8 days compared to the controls (Vargas-Sánchez et al., 2014).

According to Yaman (2023), chicken meat enriched with water extract propolis, 5 % and 10 % (WEP) showed a significantly lower amount of bacteria count (6.28 log CFU/g) compared to control (7.87 log CFU/g). Besides, the author also mentioned that 5 % of WEP showed the lowest antibacterial and antioxidant activity on chicken meat while 15 % of WEP recorded great microbial properties but the lowest score on customer acceptability due to undesirable chicken meat odour and colour. A study investigated by Gedikoğlu (2022) noticed that the growth of mesophilic bacteria and Enterobacteriaceae count on ground beef meatballs were significantly low, which were 2.42 log CFU/g and 2.24 log CFU/g, respectively when treated with water extract propolis (WEP) compared to controls.

Hence, this finding demonstrated that propolis extract exhibited a strong antimicrobial property against foodborne pathogens, preventing them from contaminating red meat. Moreover, propolis extract can also be considered as a potential natural preservative that offers improvement in the shelf life of red meat products when kept in cold storage.

4. Conclusion

The samples exhibit DPPH scavenging activity, TFC, and TPC concentrations in the following order: *H. fimbriata*>*T. apicalis*>*T. binghami*. Three Malaysian stingless bee propolis ethanolic extracts were used in this study enabling the correlation between the bioactive compounds identified in the propolis extracts with pharmacological characteristics exhibited by them. Interestingly, the propolis extract used may be utilized as an additive for food preservation as the results showed that it was able to significantly control the growth of foodborne bacteria in raw beef after 14 days during cold storage when supplemented with the propolis extract compared to the control groups. Propolis utilized in this investigation offers potent bioactive compounds capable of supporting various biological functions. Hence, several prosperous and promising

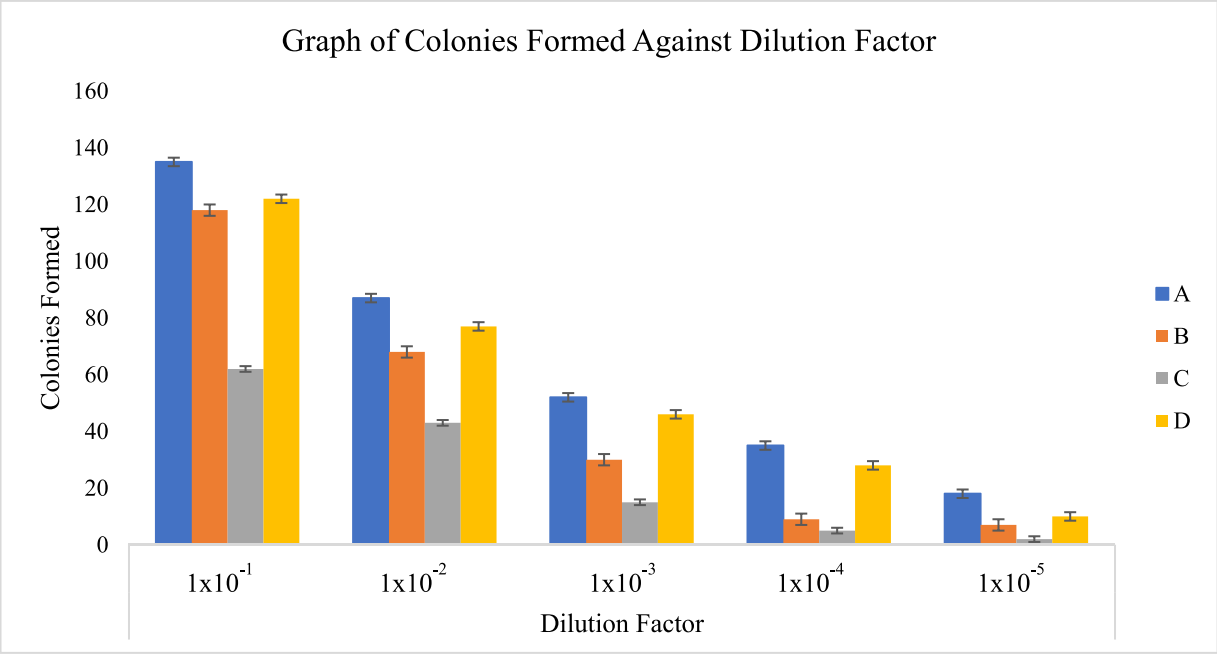

Fig. 2. Graph of colonies formed against beef samples (A, B, C, and D) with different dilution factors (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}).

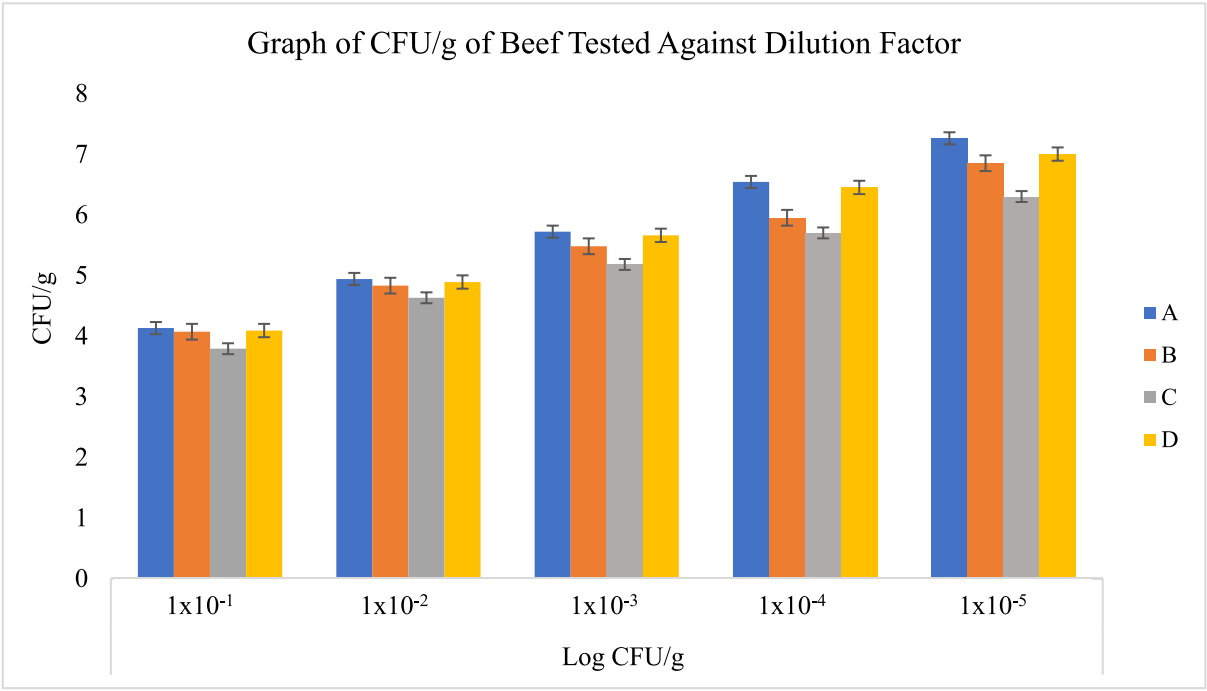

Fig. 3. Graph of CFU/g of beef tested against different dilution factors.

futures for propolis applications in packaging and food can be explored. Firstly, the expansion of its use as a food additive is hindered by its bitter taste and unpleasant odour, which allows it to be used only in small proportions. This problem could be overcome by conducting modern research that provides propolis in an acceptable form of encapsulated product or composites to reduce unpalatable taste and smell. Furthermore, chemical analysis of the qualitative and quantitative contents of the propolis was not carried out in the current study to identify which chemicals were involved in the preservation action and their respective concentration in the extract. This step is crucial in standardizing propolis extract for future use in food industries. Malaysia is endowed with a wide range of plant species, and the diversity of floral sources that can make it challenging to evaluate the constituents of propolis. For this reason, identifying particular resin sources from various trees and vegetation is crucial to comprehending propolis extracts' chemistry.

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Ethical statement – studies in humans and animals

*No test on animals or humans been done for this study.

CRediT authorship contribution statement

Nur Ayuni Mohd Hanapiah: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sharifah Nur Amalina Syed Salleh:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Wan Lutfi Wan Johari:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Noranizan Mohd Adzahan:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Conceptualization. **Normala Halimoon:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization. **Nurul Huda Osman:** Writing – review & editing, Validation, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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