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Improving the Shelf Life and Quality of Minced Beef by Cassia Glauca Leaf Extracts during Cold Storage

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Abstract: Minced beef is a popular meat product due to its low price and superior nutritional value. The contamination of minced beef is a significant risk for the worldwide meat market. Both natural and synthetic preservatives are used to expand the shelf life and improve the quality properties of meat. The harmful effects of synthetic preservatives make natural preservatives more appealing. Therefore, this research was performed to study the impact of different concentrations of Cassia glauca leaf extract (CGE) on increasing the shelf life of minced beef. Seventy-two minced beef samples were divided into control, 0.25, 0.5, and 1% w/w CGE treated groups. The control and treated samples were kept at 3 ± 1 °C in the refrigerator for 15 days. Minced beef samples' sensory, chemical, and microbiological properties were assessed every three days. The gained results showed that the CGE addition effectively decreased the microbial count and maintained the minced beef's sensory and chemical quality. Additionally, CGE extended the shelf life of minced meat up to 15 days under the proper refrigeration condition compared to the control group, which decomposed after the sixth day of refrigeration. Our study suggested that CGE could be used as a natural preservative for refrigerated minced meat.

Keywords: minced beef; natural preservative; shelf life; Cassia glauca leaf extract

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1. Introduction

Meat and meat products are the requisite food for many consumers in developing and developed countries, owing to their high protein, vitamin, and mineral contents [1]. It is an essential dietary ingredient that enhances adequate growth and development in children and the welfare and health of adults and seniors [2].

Meats with different nutrients are usually vulnerable to lipid oxidation and microbial contamination [3]. Meat spoilage negatively impacts public health and quality, affecting the economy [4]. Several synthetic preservatives are applied to reduce oxidative reactions, hinder microbial growth, and subsequently expand the shelf life of minced meat [5]. Meat is preserved not only by the addition of antioxidant substances but by the long-known and used-commonly-nowadays addition of salt. This is mainly used for the production of dry-cured hams [6–8]. Another method is the addition of organic acids and their salts [9,10].

The upward realization of human health and the correct fear of artificial compounds have created the necessity to explore the usefulness of natural preservatives in expanding the shelf life of stored meat products besides maintaining their safety and quality [11].

Multiple investigations have reported the antioxidant and antibacterial action of natural product-based preservatives in the meat field [12].

Cassia glauca belongs to the Caesalpiniaceae sub-family and Fabaceae family. This ornamental plant is rich in phenolic acids and anthraquinones, including Apigenin, Quercetin, Quercetin-3-O-β-D-glucopyranoside, Luteolin, Rutin, and Kaempferol-3-O-rutinoside [13]. Various biological activities of phenols and anthraquinones have been reported, including antioxidant, antibacterial, antigenotoxic, and antimutagenic properties [14].

The ethanolic extract of Cassia glauca exhibits high antioxidant and antimicrobial properties, particularly against *staphylococcus, Enterococcus faecalis, Klebsiella, Escherichia coli, Aspergillus fumigata*, and *Candida Albicans* [15]. Although minimal data are available about Cassia glauca as a meat preservative, it has been reported for its various pharmaceutical activities, such as antidiabetic and anticarcinogenic properties [16–18].

The high phenolic content and the antioxidant, antibacterial, and antifungal activities characterizing Cassia glauca increased our expectancy about using the plant extract as a natural meat preservative. The purpose of this study was to investigate the effect of the Cassia glauca leaf extract (CGE) at three concentrations (0.25%, 0.5%, and 1%) on the sensory attributes and chemical and microbiological quality of minced meat to determine whether or not it has the potential to be used as a natural preservative for minced meat. At the same time, it is being kept in the refrigerator.

2. Materials and Methods

2.1. Extraction of Cassia Glauca Leaf Extract (CGE)

Cassia glauca leaves were thoroughly washed with tap water, followed by distilled water, and dried in the shade for around 30 days [19]. The dried leaves were crushed into a coarse powder using a blender [20]. The dried leaf powder was soaked for three days in 100% absolute ethanol at 7.5 times the weight of the dried leaf powder [21]. The extract was left for one day to stand to form the sediment; then, the extract was filtered. A rotary evaporator concentrated the filtered extract at a temperature of 45 °C to obtain a thick ethanolic Cassia glauca extract [21]. The obtained extract was used in three concentrations, 0.25%, 0.5%, and 1% (w/w), and was added to minced meat samples

2.2. Preparation of Minced Beef Samples

A total of 72 minced beef samples of Egyptian Baladi cattle (200 g each) were purchased from butcher shops in El Gharbia governorate, Egypt, packed in sterile polyethylene bags [22]. The samples were rapidly transferred in an iced box to the food and feed safety lab at the faculty of veterinary medicine Damanhur university, Egypt. Minced beef samples were divided into four groups; the first one was control (without any treatment), and the other three groups were mixed with 0.25%, 0.5%, and 1% (w/w) of ethanolic Cassia glauca leaf extracts (18 samples in each group). The minced beef samples were homogenized in a stainless steel blender. Ground beef was mixed with latex-gloved hands. Treated samples were mixed well with CGE (0.25%, 0.5%, and 1%). The treated and control samples were shaped into small balls by hand and packed in polyethylene bags. All samples were stored for 15 days in the refrigerator (3 ± 1 °C). A sample from each group was analyzed every 3 days, on the experiment's 0, 3rd, 6th, 9th, 12th, and 15th day [23].

2.3. Sensory Evaluation of Minced Beef Samples

During the 15 days of preservation in the refrigerator, the sensory quality of the minced beef was based on a 9-point scale [24]. A panel of 18 (adults, 24–50 years old, untrained) performed the sensory analysis. Each group's sample (50 ± 10 g) was coded with three-digit numbers and served randomly to the panelists. Tap water between the samples was given to the panel groups to rinse their palate from the previous sample taste. The panelists were requested to record their preferences on a nine-point hedonic scale. The scale points were as follows: 7–9 "very good" quality, 4.0–6.9 "good" quality, 1.0–3.9

"spoiled". This was used for the appearance, smell, texture, taste (after cooking without salt or spices), and overall acceptability evaluation of both treated and control samples.

2.4. Chemical Analysis of Minced Beef Samples

2.4.1. pH Measurement

According to Pearson [25], the pH measurement was carried out using an electrical pH meter (Bye model 6020, USA). The pH meter was calibrated using two precisely recognized buffer solutions (pH 7.01 and 4.01). Briefly, 10 g of each minced beef sample was blended in 10 mL of neutralized distilled water, left at room temperature for 10 min with shaking, and filtered.

2.4.2. Determination of the Total Volatile Basic Nitrogen Content (TVBN) (mg/100 g)

TVBN was evaluated rendering to EOS 63/9 [26]. Briefly, 10 g of each minced beef sample was added to 300 mL of distilled water in a Kjeldahl flask, thoroughly mixed, and then 2 g of magnesium oxide was added. The solution was boiled, the distillate was collected in a receiving flask containing 25 mL of 2% boric acid, and a few drops of the indicator were added. Titration of TVBN was performed by H_2SO_4 0.1 M until a faint pink color was obtained. Consequently, TVBN was calculated from the following formula: TVBN mg/100 g = R $\times$ 14, where R is the volume of H_2SO_4 exhausted in titration.

2.4.3. Determination of Thiobarbituric Acid (TBA)

The TBA number was performed according to EOS 63/10 [26]. Briefly, 10 g of each sample was mixed with 50 mL of distilled water. In a distillation flask, 2.5 mL of hydrochloric acid (diluted in 47.5 water) and small pieces of antifoaming agents were added. The flask was heated to collect 50 mL of distillate within 10 min of the onset of boiling. Consequently, 5 mL of the distillate was conveyed in a tube with a cover and mixed with 5 mL of prepared thiobarbituric acid. The tube was boiled in a water bath for 35 min, and then cooled under tap water for 10 min. A spectrophotometer (Unicam 969 AAS; Milton Roy Co., Rochester, NY, USA) was used to measure the absorbance of the sample under wavelength 538 nm. The TBA number was computed as mg malonaldehyde per kg of the sample by multiplying the absorbance value by 7.8.

2.4.4. Determination of Peroxide Value (PV)

PV was determined according to the method recommended by Rahman et al. [27]. Accurately, 3 g of each sample was heated within a glass stopper Erlenmeyer flask in a water bath at 60 °C for 3 min for fat melting. Acetic acid-chloroform solution (3:2 *v*/*v*, 30 mL) was added into the flask with proper agitation for 3 min to liquefy the fat. The mixture was filtered, and 0.5 mL of the saturated potassium iodide solution was added to the filtrate; a starch solution was added as an indicator. The solution was titrated with sodium thiosulfate standard solution. Peroxide value (PV) was expressed as milliequivalent peroxide per kilogram of the sample by the following equation: PV ($meqO_2/Kg$) = (S $\times$ N)/W $\times$ 100, where S is the volume of titration (mL), N is the normality of sodium thiosulfate solution (N = 0.01), and W is the weight of the sample (g).

2.5. Microbiological Examination of Minced Beef Samples

Under complete aseptic conditions, ten grams of each sample was weighed and homogenized with 90 mL of sterile peptone water (0.1%) in a stomacher (AES chemunex-AESAP 1064) for 1 min at 1000 rpm. A proper tenfold serial dilution was prepared [28]. The total aerobic bacterial count (TBC) was assessed on plate count agar after incubation at 37 °C for 48 h [29]. Psychrotrophic bacterial count (PBC) was assayed by pouring, using plate count agar with incubation at 7 °C for ten days (81001-5-1 2003). Enterobacteriaceae count was determined after distribution on violet red bile glucose agar with incubation at 30 °C for 24 h [30]. The staphylococcal count was enumerated using Baird parker agar medium after incubation at 37 °C for 48 h [31]. The total mold and yeast count was demonstrated using Sabouraud's dextrose agar at 25 ± 1 °C for 5 to 7 days [32]. All used media in the microbiological analyses were purchased from HI Media Laboratories, Marg, Mumbai-400086, India. The results were expressed as log10 CFU/g per sample.

2.6. Statistical Analysis

All experiments were conducted in triplicate. Statistical Program for Social Science (SPSS) was used to analyze the outcomes. The obtained data of sensory attributes, chemical and microbiological evaluation from different treatments, and storage times were submitted for analysis of variance (one-way ANOVA). Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage, and the significance of the data was accepted at the probability of $p \leq 0.05$.

3. Results

3.1. Sensory Evaluation of Minced Beef Samples

Table 1 shows that the sensory attributes of minced beef were significantly increased by different concentrations of CGE (0.25%, 0.5%, and 1%) compared to the control during cold storage (3 ± 1 °C) for 15 days. The addition of CGE at 0.25, 0.5, and 1% maintained the sensory properties for 9, 12, and 15 days, respectively. The control samples were spoiled after the sixth day of cold storage.

3.2. Chemical Analysis of Minced Beef Samples

3.2.1. Hydrogen Ion Concentration (pH) of Minced Beef Samples

Minced beef combined with an increasing concentration of CGE showed significantly decreasing pH values compared to control samples as the storage period proceeded (Table 2). The increasing pH rate was faster in control samples compared to CGE-containing ones (0.25%, 0.5%, and 1% CGE) during cold storage (3 ± 1).

3.2.2. Total Volatile Nitrogen of Minced Beef Samples

Table 3 shows that different concentrations of CGE showed slightly decreased TVBN content in treated minced meat samples compared to the control. The control group exceeded the permissible limit by the sixth day of storage (21.92 ± 1.24 mg/100). It became unfit compared to the minced beef meat group incorporated with 1% CGE, which became unfit (20.07 ± 0.61 mg/100) after 15 days of storage at 3 ± 1 °C. Our results revealed that CGE could reduce protein decomposition and decrease TVN values.

3.2.3. Thiobarbituric Acid Reactive Substances of Minced Beef Samples

Table 4 shows that different concentrations of CGE showed a slight decrease in TBARS content value in treated minced meat samples compared to the control group by day 6 (1.07 ± 0.13 mg MDA/kg). Our findings revealed that Cassia glauca leaf extracts can reduce lipid oxidation and decrease the TBARS values.

3.2.4. Peroxide Values of Minced Beef Samples (PV)

Table 5 shows that different concentrations of CGE showed slightly decreased peroxide values in treated minced meat samples compared to the control samples. The addition of CGE in minced beef delayed lipid oxidation development and decreased the peroxide values of treated samples compared to the untreated control samples.

Table 1. Changes in sensory attributes of minced beef samples treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during cold storage at 3 ± 1 °C (mean ± standard deviation "SD").

Sensory Attributes Storage (Days)	Cassia Glauca Leaf Extract Concentrations (%)			
	Control	0.25%	0.50%	1.0%
Appearance				
0 day	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}	8.7 ± 0.6^{Aa}	8.7 ± 0.6^{Aa}
3 day	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}	8.7 ± 0.6^{Aa}	8.7 ± 0.6^{Aa}
6 day	4.3 ± 0.6^{Ba}	7.3 ± 0.6^{Bb}	7.7 ± 0.6^{Ab}	8.0 ± 0.0^{Ab}
9 day	Decomposed	5.3 ± 1.2^{Ca}	6.7 ± 0.6^{Aa}	6.7 ± 0.6^{Ba}
12 day	Decomposed	Decomposed	4.3 ± 0.6^{Ba}	5.7 ± 0.6^{Cb}
15 day	Decomposed	Decomposed	Decomposed	4.7 ± 0.6^{D}
Smell				
0 day	9.0 ± 0.0^{Aa}	8.7 ± 0.6^{Aa}	8.3 ± 0.6^{Aa}	8.0 ± 0.0^{Aa}
3 day	8.0 ± 1.0^{Aa}	8.7 ± 0.6^{Aa}	8.0 ± 0.0^{Aa}	7.7 ± 0.6^{Aa}
6 day	5.0 ± 1.0^{Ba}	6.7 ± 0.6^{Bb}	7.3 ± 0.6^{Ab}	7.7 ± 0.6^{Ab}
9 day	Decomposed	4.3 ± 0.6^{Ca}	6.3 ± 0.6^{Bb}	7.0 ± 0.0^{Bb}
12 day	Decomposed	Decomposed	4.0 ± 1.0^{Ca}	5.3 ± 0.6^{Ca}
15 day	Decomposed	Decomposed	Decomposed	3.7 ± 0.6^{D}
Texture				
0 day	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}
3 day	5.7 ± 0.6^{Ba}	8.7 ± 0.6^{Ab}	9.0 ± 0.0^{Ab}	9.0 ± 0.0^{Ab}
6 day	4.3 ± 0.6^{Ca}	4.7 ± 0.6^{Ba}	8.0 ± 0.0^{Bb}	8.3 ± 0.6^{Ab}
9 day	Decomposed	3.7 ± 0.6^{Ca}	5.7 ± 0.6^{Cb}	7.7 ± 0.6^{Bc}
12 day	Decomposed	Decomposed	3.7 ± 0.6^{Da}	5.3 ± 0.6^{Cb}
15 day	Decomposed	Decomposed	Decomposed	3.7 ± 0.6^{D}
TASTE				
0 day	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}	8.3 ± 0.6^{Ab}
3 day	7.3 ± 0.6^{Ba}	8.7 ± 0.6^{Ab}	8.3 ± 0.6^{Bb}	8.0 ± 0.0^{Ab}
6 day	5.7 ± 0.6^{Ca}	7.0 ± 0.0^{Bb}	8.0 ± 0.0^{Bc}	8.0 ± 0.0^{Ac}
9 day	Decomposed	4.7 ± 0.6^{Ca}	6.3 ± 0.6^{Cb}	7.3 ± 0.6^{Bb}
12 day	Decomposed	Decomposed	4.0 ± 0.0^{Da}	5.3 ± 0.6^{Cb}
15 day	Decomposed	Decomposed	Decomposed	3.3 ± 0.6^{D}
Overall acceptability				
0 day	9.0 ± 0.0^{Aa}	9.0 ± 0.0^{Aa}	8.3 ± 0.6^{Aa}	8.3 ± 0.6^{Aa}
3 day	6.3 ± 0.6^{Ba}	6.7 ± 0.6^{Ba}	7.7 ± 0.6^{Ab}	8.0 ± 0.0^{Aa}
6 day	4.0 ± 1.0^{Ca}	5.3 ± 0.6^{Ca}	6.3 ± 0.6^{Bb}	7.3 ± 0.6^{Bb}
9 day	Decomposed	3.7 ± 0.6^{Da}	4.7 ± 0.6^{Ca}	6.3 ± 0.6^{Cb}
12 day	Decomposed	Decomposed	3.3 ± 0.6^{Da}	4.7 ± 0.6^{Db}
15 day	Decomposed	Decomposed	Decomposed	3.7 ± 0.6^{E}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

Table 2. The pattern of the pH values of minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	Cassia Glauca Leaf Extract Concentrations (%)			
	Control	0.25%	0.50%	1.0%
0 day	5.75 ± 0.30 ^{Aa}	5.73 ± 0.06 ^{Aa}	5.72 ± 0.06 ^{Aa}	5.71 ± 0.06 ^{Aa}
3 day	6.30 ± 0.08 ^{Ba}	5.87 ± 0.08 ^{Ab}	5.82 ± 0.07 ^{Ab}	5.77 ± 0.05 ^{Ab}
6 day	6.79 ± 0.13 ^{Ca}	6.10 ± 0.13 ^{Bb}	5.98 ± 0.08 ^{Ab}	5.84 ± 0.06 ^{Ab}
9 day	Decomposed	6.47 ± 0.28 ^{Ca}	6.28 ± 0.18 ^{Bb}	6.08 ± 0.14 ^{Bb}
12 day	Decomposed	Decomposed	6.57 ± 0.28 ^{Ca}	6.32 ± 0.15 ^{Cb}
15 day	Decomposed	Decomposed	Decomposed	6.47 ± 0.13 ^{C}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

Table 3. The pattern of the total volatile nitrogen (TVN) (mg%) of the minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	Cassia Glauca Leaf Extract Concentrations (%)			
	Control	0.25%	0.50%	1.0%
0 day	2.96 ± 0.18 ^{Aa}	2.89 ± 0.17 ^{Aa}	2.85 ± 0.16 ^{Aa}	2.79 ± 0.17 ^{Aa}
3 day	13.31 ± 0.48 ^{Ba}	6.21 ± 0.33 ^{Bb}	5.87 ± 0.25 ^{Bb}	5.72 ± 0.26 ^{Bb}
6 day	21.92 ± 1.24 ^{Ca}	10.96 ± 0.72 ^{Cb}	8.89 ± 0.38 ^{Cc}	8.30 ± 0.57 ^{Cc}
9 day	Decomposed	17.41 ± 0.90 ^{Da}	14.77 ± 0.74 ^{Db}	13.06 ± 0.86 ^{Dc}
12 day	Decomposed	Decomposed	19.96 ± 1.10 ^{Ea}	17.22 ± 0.92 ^{Eb}
15 day	Decomposed	Decomposed	Decomposed	20.07 ± 0.61 ^{F}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

Table 4. The pattern of thiobarbituric acid (TBA) (mg/kg) of minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	Cassia Glauca Leaf Extract Concentrations (%)			
	Control	0.25%	0.50%	1.0%
0 day	0.07 ± 0.02 ^{Aa}	0.07 ± 0.01 ^{Aa}	0.06 ± 0.00 ^{Aa}	0.06 ± 0.01 ^{Aa}
3 day	0.53 ± 0.08 ^{Ba}	0.27 ± 0.03 ^{Bb}	0.22 ± 0.03 ^{Bb}	0.17 ± 0.02 ^{Bb}
6 day	1.07 ± 0.13 ^{Ca}	0.45 ± 0.07 ^{Cb}	0.38 ± 0.04 ^{Cb}	0.27 ± 0.07 ^{Bb}
9 day	Decomposed	0.78 ± 0.06 ^{Da}	0.69 ± 0.07 ^{Da}	0.55 ± 0.08 ^{Cb}
12 day	Decomposed	Decomposed	0.87 ± 0.09 ^{Ea}	0.75 ± 0.09 ^{Da}
15 day	Decomposed	Decomposed	Decomposed	0.88 ± 0.06 ^{E}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

Table 5. The pattern of peroxide value (PV) (mEq/kg) of the minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	Cassia Glauca Leaf Extracts Concentrations (%)			
	Control	0.25%	0.50%	1.0%
0 day	0.11 ± 0.02 ^{Aa}	0.10 ± 0.01 ^{Aa}	0.09 ± 0.01 ^{Aa}	0.09 ± 0.02 ^{Aa}
3 day	0.60 ± 0.08 ^{Ba}	0.34 ± 0.03 ^{Bb}	0.29 ± 0.04 ^{Bb}	0.23 ± 0.04 ^{Bb}
6 day	1.18 ± 0.13 ^{Ca}	0.54 ± 0.07 ^{Cb}	0.45 ± 0.05 ^{Cb}	0.36 ± 0.07 ^{Cb}
9 day	Decomposed	0.87 ± 0.06 ^{Da}	0.80 ± 0.05 ^{Da}	0.63 ± 0.07 ^{Db}
12 day	Decomposed	Decomposed	0.97 ± 0.09 ^{Ea}	0.83 ± 0.08 ^{Ea}
15 day	Decomposed	Decomposed	Decomposed	0.99 ± 0.07 ^{F}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

3.3. Microbiological Examination of Minced Beef with CGE

3.3.1. Total Aerobic Bacterial Count (TABC) of Minced Beef Samples

Table 6 shows that, at day zero, the control samples had the highest initial total bacterial count (5.70 ± 0.03 log10 cfu/g) followed by 0.25% CGE treated sample (5.66 ± 0.03 log10 cfu/g). In contrast, the lowest initial total bacterial presented with 0.5% and 1% CGE treated samples (5.63 ± 0.05 and 5.61 ± 0.02 log10 cfu/g, respectively). Interestingly, samples treated with 0.25%, 0.5%, and 1% CGE showed slightly decreased TABC value compared to the control samples. The control group exceeded the acceptable level by day 6 of storage compared to the minced meat samples treated with 0.25%, 0.5, and 1%, which became unacceptable after 9, 12, and 15 days of storage at 3 ± 1 °C, respectively. Our results showed that CGE positively impacted the total aerobic plate count by increasing the CGE in minced meat from 0.25% to 0.5% and 1% and increasing the shelf life of minced meat from 6 to 9, 12, and 15 days, respectively.

Table 6. Pattern of the aerobic bacterial count (log10 cfu/g) in minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	**Cassia Glauca Leaf Extracts Concentrations (%)**			
	Control	**0.25%**	**0.50%**	**1.0%**
0 day	5.70 ± 0.03 ^{Aa}	5.66 ± 0.03 ^{Aa}	5.63 ± 0.05 ^{Aa}	5.61 ± 0.02 ^{Aa}
3 day	6.00 ± 0.02 ^{Ba}	5.90 ± 0.01 ^{Bb}	5.78 ± 0.04 ^{Bc}	5.74 ± 0.04 ^{Bc}
6 day	6.45 ± 0.01 ^{Ca}	6.00 ± 0.01 ^{Cb}	5.97 ± 0.03 ^{Cc}	5.88 ± 0.04 ^{Cd}
9 day	Decomposed	6.46 ± 0.02 ^{Da}	6.02 ± 0.03 ^{Db}	6.00 ± 0.04 ^{Dc}
12 day	Decomposed	Decomposed	6.47 ± 0.00 ^{Ea}	6.04 ± 0.05 ^{Eb}
15 day	Decomposed	Decomposed	Decomposed	6.44 ± 0.03 ^{F}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

3.3.2. Total Psychrotrophic Count (TPC) of Minced Beef Samples

Results obtained in Table 7 show that CGE-containing samples revealed significantly lower TPC throughout the storage period compared to untreated controls. In control samples, TPC increased from 5.54 ± 0.02 log10 cfu/g at zero days of storage to 6.48 ± 0.00 log10 cfu/g at day 6 of storage. In CGE-treated samples (0.25%, 0.5%, and 1%), TPC increased from 5.51 ± 0.02, 5.50 ± 0.03, and 5.49 ± 0.01 log10 cfu/g at zero days of storage to 6.21 ± 0.01, 5.95 ± 0.01, and 5.89 ± 0.01 log10 cfu/g at day 6 of storage, respectively.

Table 7. Pattern of the psychrophilic count (log10 cfu/g) in minced beef treated with ethanolic Cassia glauca leaf extracts. (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	**Cassia Glauca Leaf Extract Concentrations (%)**			
	Control	**0.25%**	**0.50%**	**1.0%**
0 day	5.54 ± 0.02 ^{Aa}	5.51 ± 0.02 ^{Aa}	5.50 ± 0.03 ^{Aa}	5.49 ± 0.01 ^{Aa}
3 day	6.21 ± 0.03 ^{Ba}	5.78 ± 0.03 ^{Bb}	5.69 ± 0.02 ^{Bc}	5.61 ± 0.03 ^{Bd}
6 day	6.48 ± 0.00 ^{Ca}	6.21 ± 0.01 ^{Cb}	5.95 ± 0.01 ^{Cc}	5.89 ± 0.01 ^{Cd}
9 day	Decomposed	6.48 ± 0.00 ^{Da}	6.23 ± 0.01 ^{Db}	6.00 ± 0.01 ^{Dc}
12 day	Decomposed	Decomposed	6.47 ± 0.00 ^{Ea}	6.25 ± 0.01 ^{Eb}
15 day	Decomposed	Decomposed	Decomposed	6.48 ± 0.00 ^{F}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

3.3.3. Total Enterobacteriaceae Count (TEC) of Minced Beef Samples

Table 8 shows that the initial Enterobacteriaceae count was the highest in the control sample (3.51 ± 0.01 log10 cfu/g) followed by 0.25% CGE-treated samples (3.50 ± 0.02

log10 cfu/g), 0.5% CGE-treated samples (3.49 ± 0.02 log10 cfu/g), and 1% CGE-treated samples (3.48 ± 0.01 log10 cfu/g). Different concentrations of CGE (0.25, 0.5, and 1%) incorporated with minced samples showed a slight decrease in TEC compared to the control sample. The control minced beef samples started decomposition on day 6 of storage, while decomposition within the treated samples with 0.25 %, 0.5%, and 1% of CGE was delayed to the 9th and 12th days of storage, respectively.

Table 8. Pattern of the Enterobacteriaceae count (log10 cfu/g) in minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	Cassia Glauca Leaf Extracts Concentrations (%)			
	Control	**0.25%**	**0.50%**	**1.0%**
0 day	3.51 ± 0.01^{Aa}	3.50 ± 0.02^{Aa}	3.49 ± 0.02^{Aa}	3.48 ± 0.01^{Aa}
3 day	4.03 ± 0.02^{Ba}	3.90 ± 0.02^{Bb}	3.73 ± 0.04^{Bc}	3.70 ± 0.01^{Bc}
6 day	4.48 ± 0.00^{Ca}	4.08 ± 0.01^{Cb}	3.94 ± 0.01^{Cc}	3.90 ± 0.01^{Cd}
9 day	Decomposed	4.48 ± 0.00^{Da}	4.09 ± 0.02^{Db}	3.97 ± 0.02^{Dc}
12 day	Decomposed	Decomposed	4.48 ± 0.00^{Ea}	4.11 ± 0.01^{Eb}
15 day	Decomposed	Decomposed	Decomposed	4.48 ± 0.00^{F}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

3.3.4. Total Staphylococcal Count (TSC) of Minced Beef Samples

The obtained results in Table 9 show that the initial staphylococcal count of the control (3.52 ± 0.02 log10 cfu/g) and 0.25%, 0.5%, and 1% ECG-treated samples (3.50 ± 0.03, 3.50 ± 0.02, and 3.48 ± 0.01 log10 cfu/g, respectively) were similar. The incorporation of different CGE concentrations (0.25, 0.5, and 1%) within minced beef samples showed a slightly decreased TSC compared to the control sample, which initiated decomposition on the 6th day. In contrast, the treated samples with 0.25, 0.5%, and 1% ECG began decomposition on the 9th, 12th, and 15th days of storage, respectively. Our findings revealed that the ECG had a significant role in decreasing the count of Staphylococci.

Table 9. Pattern of the staphylococcal count (log10 cfu/g) in minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	Cassia Glauca Leaf Extract Concentrations (%)			
	Control	**0.25%**	**0.50%**	**1.0%**
0 day	3.52 ± 0.02^{Aa}	3.50 ± 0.03^{Aa}	3.50 ± 0.02^{Aa}	3.48 ± 0.01^{Aa}
3 day	3.67 ± 0.03^{Ba}	3.63 ± 0.04^{Ba}	3.58 ± 0.03^{Bb}	3.57 ± 0.02^{Bb}
6 day	4.27 ± 0.01^{Ca}	3.95 ± 0.02^{Cb}	3.84 ± 0.02^{Cc}	3.73 ± 0.04^{Cd}
9 day	Decomposed	4.28 ± 0.01^{Da}	4.20 ± 0.01^{Db}	4.03 ± 0.02^{Dc}
12 day	Decomposed	Decomposed	4.31 ± 0.01^{Ea}	4.26 ± 0.01^{Eb}
15 day	Decomposed	Decomposed	Decomposed	4.45 ± 0.00^{F}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same columns and row, respectively.

3.3.5. Total Mold and Yeast Count of Minced Beef Samples

The results displayed in Table 10 show that the total mold and yeast counts at zero days of storage were 4.55 ± 0.02, 4.52 ± 0.02, 0.52 ± 0.02, and 4.50 ± 0.03 log10 cfu/g, for control and samples treated with CGE of 0.25%, 0.5%, and 1%, respectively. Samples treated with different concentrations of CGE (0.25, 0.5, and 1%) showed slightly decreased total mold and yeast counts compared to the control sample.

Table 10. Pattern of mold and yeast counts (log10 cfu/g) in minced beef treated with ethanolic Cassia glauca leaf extracts (0.25%, 0.5%, 1%) during the cold storage period at 3 ± 1 °C (mean ± standard deviation "SD").

Storage Period	Cassia Glauca Leaf Extract Concentrations (%)			
	Control	0.25%	0.50%	1.0%
0 day	4.55 ± 0.02^{Aa}	4.52 ± 0.02^{Aa}	4.52 ± 0.02^{Aa}	4.50 ± 0.03^{Aa}
3 day	5.06 ± 0.04^{Ba}	4.91 ± 0.02^{Bb}	4.80 ± 0.03^{Bc}	4.76 ± 0.02^{Bc}
6 day	5.37 ± 0.01^{Ca}	5.12 ± 0.02^{Cb}	4.96 ± 0.02^{Cc}	4.90 ± 0.02^{Cd}
9 day	Decomposed	5.41 ± 0.02^{Da}	5.18 ± 0.01^{Db}	5.00 ± 0.01^{Dc}
12 day	Decomposed	Decomposed	5.41 ± 0.02^{Ea}	5.23 ± 0.02^{Eb}
15 day	Decomposed	Decomposed	Decomposed	5.47 ± 0.01^{F}

Means carrying a different capital or small superscript are significantly different ($p < 0.05$) in the same column and row, respectively.

4. Discussion

The sensory attributes of minced beef were significantly increased by different concentrations of CGE (0.25%, 0.5%, and 1%). Rashed et al. [33] and Butt et al. [34] reported that Cassia glauca extracts have antioxidant activity that could cause CGE to improve the sensory quality of treated samples. Notably, the CGE addition caused a decrease in acceptability compared to the control sample at zero days. This observation was in agreement with Kumar et al. [35]. They reported that some of the natural preservatives with antioxidant activity might positively or negatively affect other quality attributes, such as sensory attributes, and eventually affect the consumer acceptability of the product. The lower consumer acceptability can be overcome by using spices that can disguise the undesirable smell or taste of CGE [36]. Minced beef combined with an increasing concentration of CGE showed significantly decreasing pH values compared to control samples as the storage period proceeded. This may be connected to the high phenolic acid content of Cassia glauca extract, which has an acidic pH [37].

Similarly, previous reports have shown that meat products formulated with plant extracts exhibited lower pH values than unformulated controls [38], suggesting that CGE enhances the pH stability of minced beef and could consequently represent a protective role against spoilage microorganisms [39]. Total volatile basic nitrogen (TVBN) content is an essential indicator for assessing the freshness of meat and meat products [35]. Conferring to the acceptable limits of TVBN in meat and meat products, which should not exceed 20 mg/100 g as recommended by EOS 1694 [36] and Tometri et al. [37], our results revealed that CGE could reduce protein decomposition and decrease TVN values. This could be caused by the high total antioxidant capacity and free radical scavenging activity of Cassia glauca extract, as mentioned by El-hashish et al. [38]. These results were supported by Srinivas et al. [40] and Gupta et al. [16], who reported that glaucous Cassia extract is an effective antioxidant. Thiobarbituric acid-reactive substance (TBARS) value measuring malondialdehyde (MDA) content, which is produced through hydroperoxides, is considered an index of the oxidation status and rancidity degree [41]; Table 4 shows that different concentrations of CGE showed a slight decrease in TBARS content value in treated minced meat samples compared to the control group, which exceeded the desirable limit of TBARS value in minced beef (under 0.9 mg MDA/kg), as inputted by EOS 1694 [41], by day 6 (1.07 ± 0.13 mg MDA/kg). This could be due to the antioxidant activity of CGE, owing to their ability to deactivate and stabilize the free radicals, as mentioned by El-hashish et al. [38] and supported by Srinivas et al. [39] and Gupta et al· [16].

Similarly, previous reports have shown that meat products treated with plant extracts exhibited lower TBARs values compared to untreated controls [37]. The reduction of peroxide values might be attributed to the phenols and flavonoids of CGE that have antioxidant activity. This agrees with several reports that showed that Cassia glauca has to be considered an effective antioxidant and a rich source of phenols and flavonoids, which possess antioxidant activity [16,42]. Therefore, the incorporation of CGE in minced beef inhibits lipid oxidation. Consequently, it extends the shelf life and improves the quality of the minced beef. According to the permissible limits recorded by EOS [42], which stated that TABC should not exceed (6 log10 CFU/g), the control group exceeded the acceptable level by day 6 of storage compared to the minced meat samples treated with 0.25%, 0.5%, and 1%, which became unacceptable after 9, 12, and 15 days of storage at 3 ± 1 °C, respectively. Our results showed that CGE positively impacted the total aerobic plate count by increasing the CGE in minced meat from 0.25% to 0.5% and 1% and increasing the shelf life of minced meat from 6 to 9, 12, and 15 days, respectively. These results agree with Fatima et al. [43] and Rashed et al. [28]. They reported that Cassia glauca leaves presented significant antimicrobial activities that may be caused by chemical compounds shown in Cassia glauca, such as phenolics and anthraquinones. Our findings revealed that the ECG significantly decreased the Enterobacteriaceae count; these results agree with Fatima et al. [43] and Rashed et al. [28], who reported that Cassia glauca extract showed antibacterial activities against some Enterobacteriaceae spp such as *Escherichia coli*. All results were below the critical limits (between 6.0–7.0 log 10 CFU/g) of TPC of minced meat, as mentioned by Córdoba-Calderón et al. [44]. These results agree with Kumar et al. [45], who reported that CGE showed antibacterial activity. Additionally, Fatima et al. [43] said that Cassia glauca leaves presented significant antimicrobial activities that may be caused by chemical compounds presented in Cassia glauca, such as phenolics and anthraquinones.

Our findings revealed that the ECG had a significant role in decreasing the count of Staphylococci. This result agrees with Kittur et al. [46]. They reported that the ethanolic extract of Cassia glauca was active against some *Staphylococcus spp (S. aureus)*, which would positively impact adjusting the microbial infections such as those caused by *Staphylococcus aureus*. Additionally, Gutiérrez-Venegas et al. [47] reported that the flavonoid compounds present in ECG, such as quercetin and rutin, showed inhibition against several microorganisms' growth, particularly *Staphylococcus aureus*. Samples treated with different concentrations of CGE (0.25, 0.5, and 1%) showed slightly decreased total mold and yeast counts compared to the control sample. These results could be attributed to the antibacterial and antifungal effects of CGE, as shown by Kittur et al. [46]. They reported that the ethanolic extract of Cassia glauca was active against some mold and yeast spp, especially *Aspergillus fumigate* and *Candida albicans*. Gutiérrez-Venegas et al. [47] reported that the flavonoid compounds in ECG, such as rutin and quercetin, showed inhibition against *Candida albicans*.

5. Conclusions

This study provides, for the first time, evidence for the possible utilization of Cassia glaucous ethanolic extract (CGE) as a natural preservative for minced beef. CGE at concentrations of 0.25%, 0.5%, and 1% improved the sensory attributes, decreased the indicated value of fat and protein oxidation, and decreased TABC, TPC, TEC, TSC, and total mold and yeast count. Therefore, CGE could perform antioxidant and antimicrobial activities and prolong the shelf life of minced beef during cold storage. Applying CGE to extend shelf life with maintaining the safety of minced beef could both please the expectation of consumers for naturally safe minced meat ingredients and add importance to this Cassia glauca plant.

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