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RESEARCH ARTICLE

Enhancing the Shelf Life of *Psidium guajava* during Post Harvesting storage by Coating with *Cinnamomum verum* Formulations

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Abstract

Guava is a nutritious fruit that could decompose during storage. In this study, (GCE F1 and GCPE F2) two formulations of cinnamon extract, Guar gum, and glycerol coating were prepared. The guavas (*Psidium guajava* L.) were selected at the stage of maturity and coated with formulations along with controls such as water and sodium benzoate. Further physiological and biochemical attributes of the quality of guava fruits were analyzed during the storage at 4°C for 5, 10 and 15 days. The results revealed that the coated guavas (GCE F1) exhibited a significant reduction in weight and firmness, low pH, and reduced usage of organic acids which contributes to high TA values, less sugar content, high antioxidant and free radical scavenging activity, with less microbial growth during the storage period. Further, the presence of bioactive compounds in the ethanolic extract of cinnamon was identified by GCMS and cytotoxicity analysis confirmed no toxic effect on L929 cell lines. The results suggest that compared to coated guavas of GCPE F2, water, and sodium benzoate, GCE F1 exhibited superior quality and enhanced shelf life during post-harvest storage.

Keywords: Cinnamon Extract, Guava, Guar Gum, Post-harvest

1. Introduction

The demand for quality fruits and vegetables has increased from the customer end. It is important to maintain the quality impute of the fruits and also control contamination during the ripening process. Guava (*Psidium guajava* L.) climacteric fruits are Neotropical fruit that is widely consumed around the world, deciduous trees, excellent sources of vitamin C, with a high profile of nutrients and sugars. During the ripening period, the fruit senescence gets accelerated which reduces the shelf life, thus deteriorating its quality. Currently, preservation methods involve the use of chemical agents in order to prolong the senescence in the fruits. However, with the impact of the chemicals and preservatives on human healthy life and the environment, there is a surge from the consumer end to shift towards a better simple, and eco-friendly choice. Various edible coating or formulation methods have been reported to enhance shelf life and improve the quality of the fruits. One such coating is cinnamon which has proven to have excellent antioxidant properties and a broad spectrum of antimicrobial activity, which in turn increases the storage period and slows the ripening process[1-3]. Therefore, there is always a demand for fresh and natural preservatives. Cinnamon oil an ideal example of essential oil is an alternative preservative in post-harvesting storage[4]. Guar gum a highly viscous polysaccharide, has also been used in edible coating.

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Guar gum is responsible for producing a thin film on the surface of fruits. This thin film maintains the quality and shelf life of food products by regulating the physical barrier such as the transfer of moisture, gases, aroma, and flavors[5,6]. It is safe for consumption, cost-effective, as well as eco-friendly, and recognized by Food and Agriculture Organization (FAO) as generally recognized as safe (GRAS). The present investigation was carried out to evaluate the physiochemical attributes and preservation quality of *Guava* fruits by coating based on *Cinnamomum verum* extracts with Guar gum.

2. Materials and Methods

For the investigation, Guava (*Psidium guajava* L.) was obtained from a commercial market, in Bengaluru. Guava fruits with green-yellowish color at the stage of maturity were selected for the study[7] considering uniformity in size and free from mechanical damages or diseases. *Cinnamomum verum* (Cinnamon), Guar gum, Sodium benzoate, and Glycerol were purchased from the local market and SRL Pvt. Ltd. respectively.

2.1 Cinnamon Extract Preparation and Formulations

The extract was done in the Soxhlet apparatus at 40°C using 3:1 ratio of cinnamon powder and solvents (ethanol, petroleum ether)[8]. The extracts were collected and kept in a dark place for evaporation. After 3 to 4 days the evaporated extracts were stored at room temperature.

The coating was prepared and formulated as follows: 5g of each solvent extract was dissolved in glycerol 35% (v/w) using a magnetic stirrer by maintaining the temperature at 60°C for 10 minutes to avoid lump formation. Further, the temperature was lowered to 45°C, thereafter 15% of Guar gum was added and volume was made up to 50ml using double distilled water. The guava fruits were washed in double distilled water and air dried. The fruits were further dipped in the formulation of cinnamon-ethanol (GCE F1) and cinnamon-petroleum ether extracts (GCPE F2). Guavas dipped in water (GDW) and sodium benzoate (GSB) were used as controls. Once the coating was completed, the fruits were stored at 4°C. Further, the experiment was designed in such a way each group (n=5) was subjected to physiological and biochemical analysis on the 5th, 10th, and 15th day of storage.

Approximately 5g of sample from each group were homogenized and the filtrate was used for each analysis.

2.2 pH

The filtrate was subjected to pH analysis, in order to determine the acidic range of controls and coated groups[9].

2.3 Titratable Acidity (TA)

For analyzing the TA, the filtrate was titrated against 0.1N NaOH in the presence of a phenolphthalein indicator (1%)[10].

2.4 Total Phenolic Content

To estimate the phenolic content, 1ml of filtrate of each group was incubated for 30 minutes at room temperature, after adding an equal amount (5 mL) of Folin's phenol solution and sodium carbonate. The spectrophotometer reading at 760 nm was recorded[11].

2.5 DPPH Assay

DPPH assay was performed to assess the degree of the antioxidant property from each group[12].

2.6 Total Sugar Content

Total sugar content was estimated using anthrone–sulphuric acid method with minor modifications[13].

2.7 Microbial Analysis

For microbial analysis, 10 ml of the filtrate was serially diluted and added to the Luria-Bertani (LB) plates in a sterile condition. The overnight incubated plates were enumerated for counting the colonies[14].

2.8 GCMS

The ethanolic extract of Cinnamon was analyzed for the presence of active compounds by JEOL GC MATE II GC-MS (VIT, Vellore, Tamil Nadu).

2.9 Cytotoxicity Study by MTT Assay

MTT assay was carried out for the ethanolic extract *of Cinnamon*[15] from Skanda Life Sciences Pvt. Ltd., DSIR-recognized R and D center, Bengaluru.

2.10 Statistical Analysis

In the present study, all the experiments were conducted in triplicates, and data analysis was performed by the ANOVA method.

3. Results

3.1 Physical Analysis of Control and Coated Fruits

The coated guava fruits were non-sticky and had a sheen appearance once the coating dried. Control fruits appeared to loose tissue firmness, shrank, and changed colour as compared to coated guava fruits of each group during the storage intervals.

Guava fruits were coated with different formulations and their physiological weight loss is represented in Table 1. Weight loss was observed in control and in coated guava fruits.

Table 1. The physiological weight loss of control and coated guavas

Samples from each group	Day 0th weight of guavas (g)	Day 5th weight of guavas (g)	Day 10th weight of guavas (g)	Day 15th weight of guavas (g)
GDW (control)	87.620	86.921	85.80	85.10
GSB (control)	88.921	88.220	87.5	86.5
GCE F1	86.551	86.220	85.922	85.55
GCPE F2	88.224	87.533	87.00	86.524

However the weight loss was drastic in control and along with a change in colour. The coated fruits possessing the effective physical barrier served as a gas and moisture barrier[16] and potentially these coated fruits showed reduced respiration rate which is associated with a decrease in their metabolic rate[17], as well as reduced water loss and decreased oxidation reactions compared to control[18]. Thus, the coating enables the fruit membrane to serve as a semipermeable layer thus preventing a fast ripening process. There was no significant weight loss in GCE F1 and GCE F2 groups.

3.2 Effects on pH and TA

pH is a measure of the concentration of free hydrogen ions in the solution. In the present study, the coated guava GCE F1, and GCPE F2 pH ranged from 3.9 to 4.2 and 3.8 to 4.0 respectively during the storage period, however, control groups showed an increase in pH which is represented in Figure 1.

During the ripening process at proper conditions, an increase in the pH and metabolic rate of fruit results in the decrease of organic acids. The tartness of guava possesses a pH of around 3-4, which is due to the presence of citric acid and less amount of malic acid and tartaric acid.

Titratable Acidity (TA) is a measure of the total amount of hydrogen ions. TA for the coated guavas GCE F1, GCPE F2 were found to be 2.2 µg/ml, 2.8 µg/ml and 1.5µg/ml, and 3.1 µg/ml, 2.9 µg/ml and 2.23µg/ml and

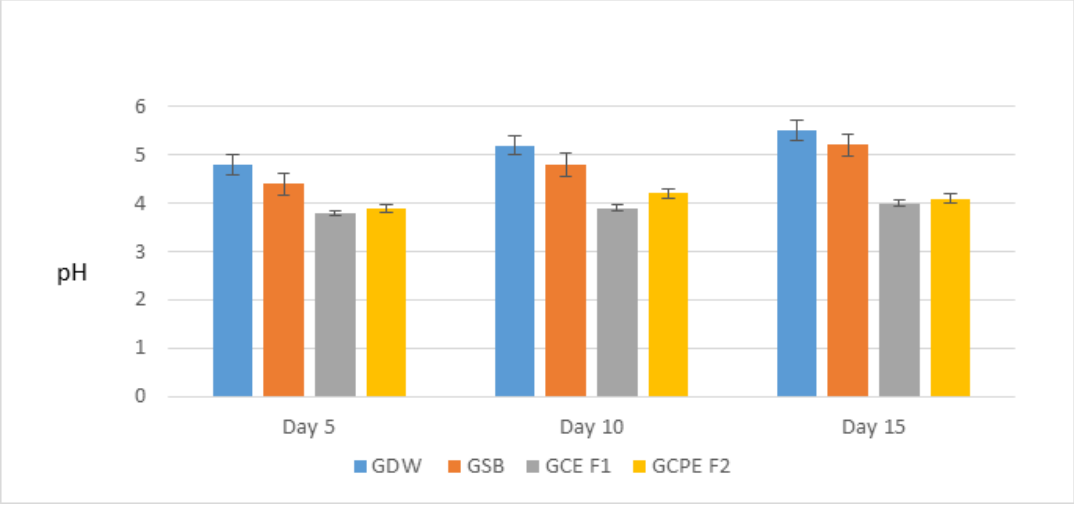

Figure 1. Effect of control and coated guava groups on pH and showed a significant difference (P>0.05).

during the storage period. There was a drastic reduction in the TA of controls as shown in Figure 2. However, in coated guava fruits, the increase in TA was suppressed due to a reduction in respiration rate. The essential substrate such as GCPE F2 malic and citric acid are decreased in acidity during respiration. The GCE F1 coated fruits showed less usage of the organic acids in the respiration process, which results in lower acid depletion in guavas. The increase in pH was paralleled by a decrease in TA seen throughout the ripening process of fruits, which is an important aspect in the concern of loss of critic acid during respiration. The reduced respiration rate might be reflected in lesser changes in pH and TA[19].

3.3 Total Phenolic Content and Antioxidant Activity

Phenolic compounds are an important aspect of the quality of fruits. The highest phenolic content was observed in the coated guavas GCEF1, and GCPEF2 were ranged from 0.688µg/ml to 0.593 µg /ml and 0.627 to 0.547µg/ml at the end of the storage period, respectively.

The reduction of the phenolic content was observed in control compared to coated guava groups as shown in Figure 3. These results are supported by earlier reports for sweet cherries coated with chitosan[18] and Aloe vera (ALV) gel coating (50% v/v) with higher biochemical quality and

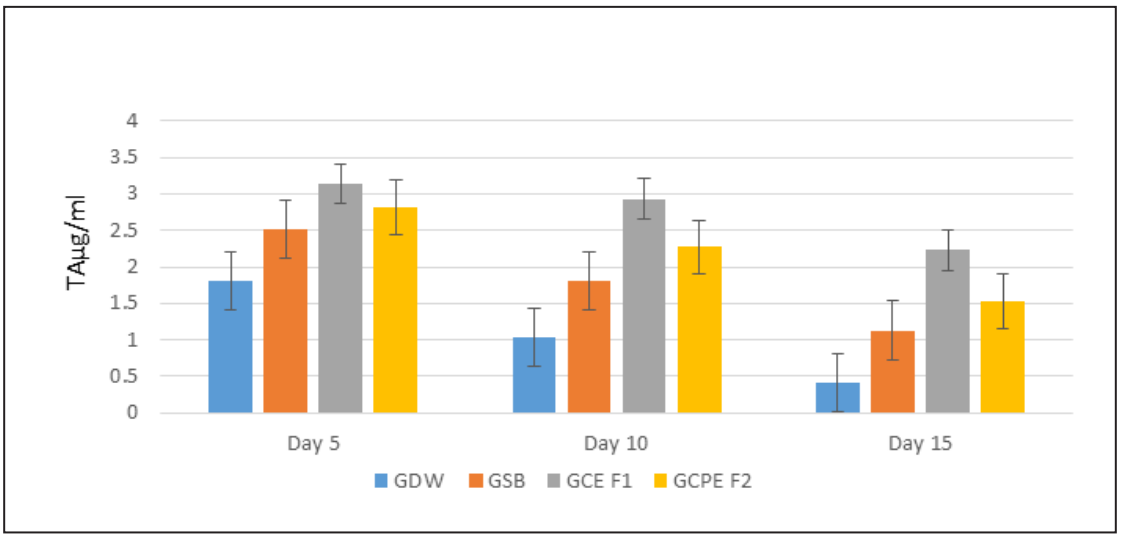

Figure 2. Effect of control and coated guava groups on TA and showed a significant difference ($p<0.05$).

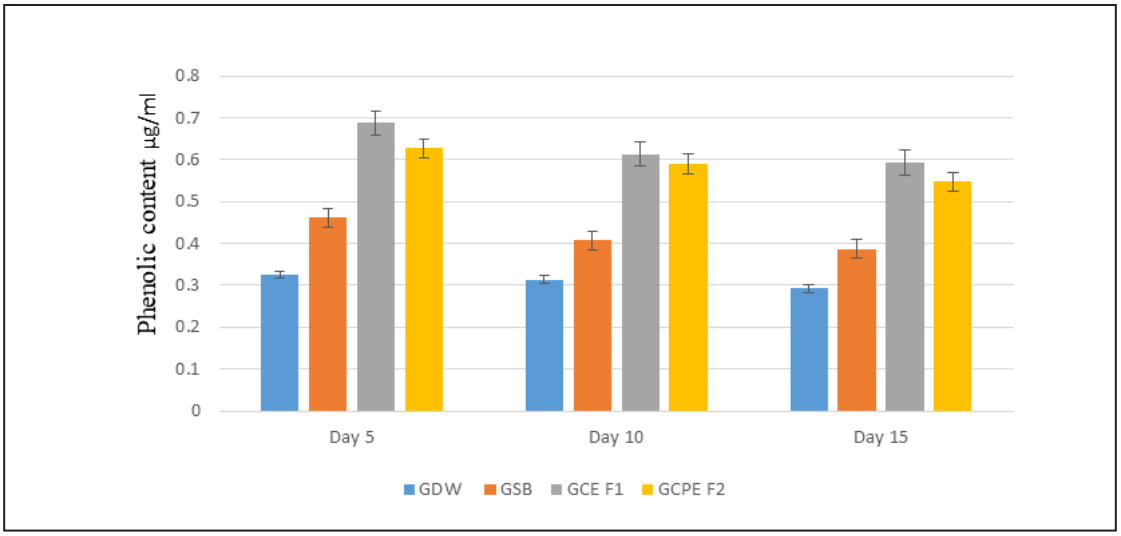

Figure 3. Effect of control and coated guava groups on phenolic content and showed a significant difference ($p<0.05$).

antioxidant enzyme activities[20]. From the observed results phenolic content of coated guavas was maintained and it is known for scavenging activity. The coated guava groups ranged from 56.4% to 54.1% and 51.7% to 44.4% represented in Figure 4. As the phenolic content was reduced in the controls, and subsequently reduced antioxidant activity was obtained. The results were supported by previous reports of cinnamon oil (CEO) which showed effective preservation of phenolic compounds and had high antioxidant potential in coating guavas[21].

3.4 Total Sugar

Total reducing sugar content in the control guava groups was found to be higher during the storage period as compared to coated guava groups, which ranged from 31.5 to 36.1 µg/ml and 56.9 to 98.6 µg/ml during the storage period, as shown in Figure 5. In the case of coated guava GCE F1 the reducing sugar content was seen in considerably increasing amounts, which could delay the aging of fruits. The presence of sucrose plays a major role in the development of fruits and in also associated with seed dispersal[22]. There are numerous studies on the metabolic-driven approaches to relating the increased sugar content during fruit ripening that determines the fruit sweetness at the harvesting stage, which is an important parameter of fruit quality[23]. However, the sugar content of the coated guavas was not increased and thus control the ripening process during post-harvest storage.

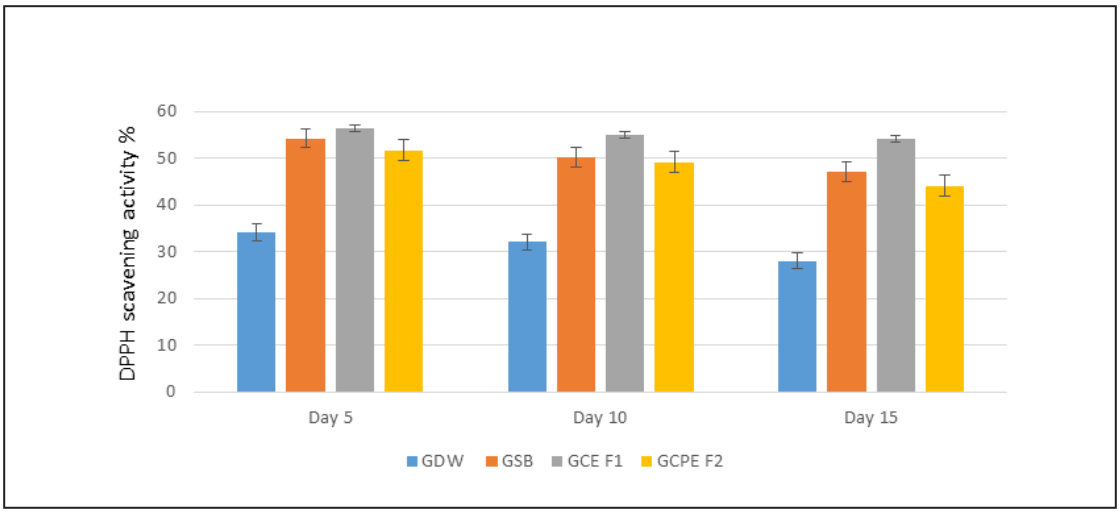

Figure 4. Effect of control and coated guava groups on free radical scavenging activity and showed a significant difference ($p<0.05$).

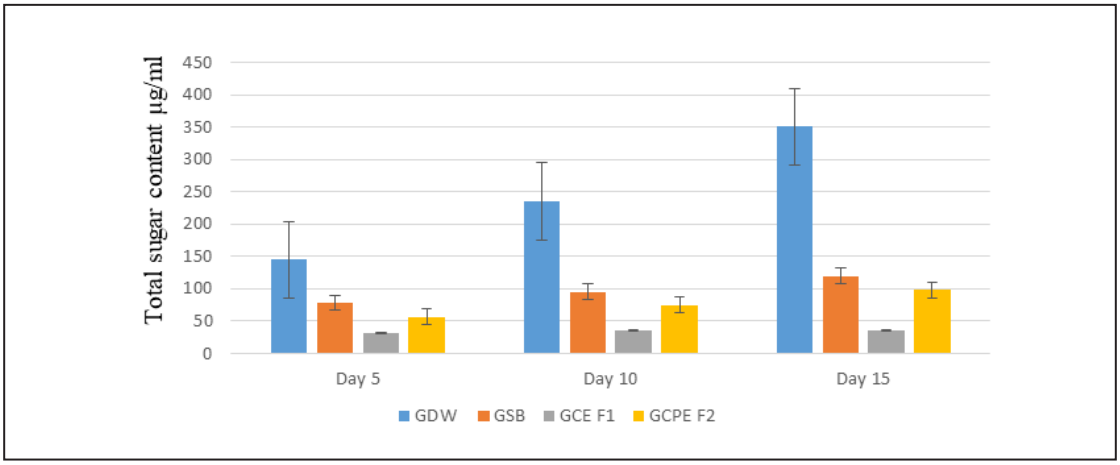

Figure 5. Effect of control and coated guava groups on total sugar content and showed a significant difference ($p<0.05$).

3.5 Microbial Analysis

The growth of mesophilic microbe counts increased with the storage period significantly for controls as compared to coated fruits. The lowest colony count was observed in the GCE F1 and followed by GCPE F2. These results proved that the coatings in guavas were acting as a barrier for gases, water, and nutrients which are necessary for microbial growth. The effective antimicrobial compound in cinnamon is a cinnamon aldehyde, which is efficient in reducing viable fungi and bacteria in fruits, especially in guava having acidic pH 3-4[24]. Cinnamon aldehyde binds to the proteins in the cell wall of microorganisms and inhibits amino acid decarboxylase enzyme[25]. Thus, the coated guavas were protected against microbial growth during the storage period.

3.6 GCMS

The coated guava GCE F1 showed better post-harvest storage quality as compared to the GCPE F2 and control groups. Therefore, the ethanolic extract was subjected to GC MS analysis. The results were compared with the retention time and their mass spectra with those of standard libraries (NIST) and the literature[26] of cinnamon ethanol extract, thus proving the presence of effective bioactive components. There are potent compounds like cinnamaldehyde (E), 2H-1-Benzopyran-2-one, and beta-Sitosterol which possess antioxidant and antimicrobial properties (Figure 6)[27,28].

3.7 Cytotoxicity Study by MTT Assay

From the above results, GCE F1 was assessed for the cytotoxicity study in L929 mouse fibroblasts cell line. There was no significant inhibition observed in the cell line as compared to the control, doxorubicin which showed IC_{50} 22.4 µM as represented in Figure 7, Table 2A and 2B. The results suggest that the ethanolic extract of cinnamon is non-cytotoxic. These findings have coincided with earlier reports of chitosan coating on the preservation of fruits (Figure 8)[29].

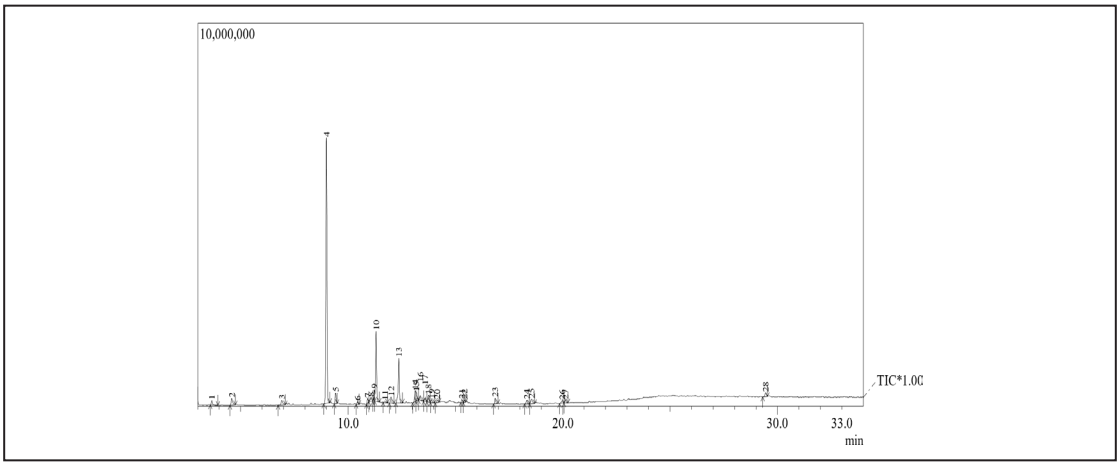

Figure 6. Chromatogram of ethanolic extract of cinnamon (GCMS-QP2010+\Data\2021\Sep\27.9.2021\1622.qgd).

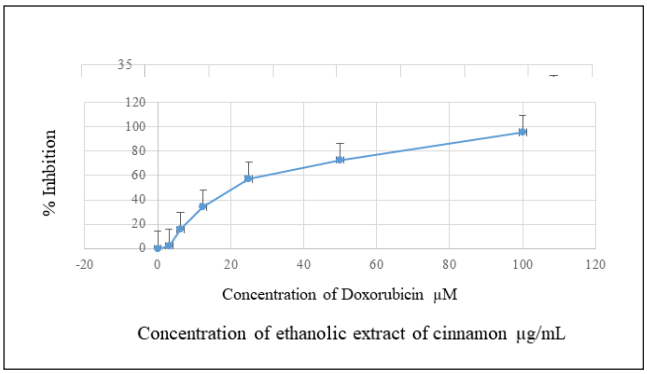

Figure 7. IC_{50} value for Doxorubicin by MTT assay using L929 cell lines.

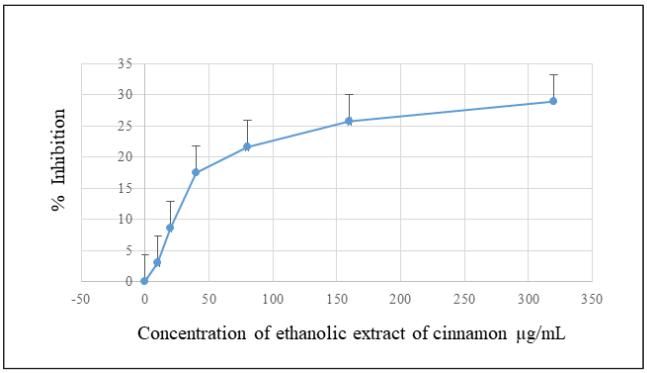

Figure 8. Percentage inhibition of Cinnamon ethanolic extract by MTT assay using L929 cell lines.

Table 2A and 2B. Inhibition percentage and IC_{50} value for the Cinnamon ethanolic extract and Doxorubicin by MTT assay using L929 cell lines

2A: L929 cell lines				
Compound name	**Conc. in µg/mL**	**OD at 590nm**	**% Inhibition**	**IC_{50} µg/mL**
Control	0	0.620	0.00	
Ethanolic extract of cinnamon	10	0.601	3.06	IC_{50} was not able to calculate due to its lesser inhibition
	20	0.567	8.55	
	40	0.511	17.58	
	80	0.486	21.61	
	160	0.460	25.81	
	320	0.440	29.03	

2B: L929 cell lines				
Sample	**Conc. in µM**	**OD at 590nm**	**% Inhibition**	**IC_{50} in µM**
Control	0	0.62	0.00	
Doxorubicin	3.125	0.608	1.94	22.4
	6.25	0.524	15.48	
	12.5	0.407	34.35	
	25	0.264	57.42	
	50	0.168	72.90	
	100	0.026	95.81	

4. Conclusion

In the present study, GCE F1 coated fruits retained the texture, with no change in colour, minimal weight loss, better antioxidant properties, antimicrobial characteristics, and non-toxic. Thus, coated fruits are eco-friendly and cost-effective. The shelf life of fruits can be enhanced without compromising their nutritional values, retaining the quality of fruits and their marketability.

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