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Original Research Article

Ginger Peel Extract-Based Edible Coating with Antioxidant and Antimicrobial Properties

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Abstract:

Edible coatings are a thin layer of a physical component that can be eaten and provides an effective barrier against oxygen, external microbes, moisture and the chemical/physical changes of the foods. The current study aimed to explore the antioxidant and antimicrobial properties of edible coating fortified with ginger peel extract (GPE). A starch-based edible coating fortified with 70% ethanolic extract of ginger peel was developed. The physical properties, antioxidant and antimicrobial properties of the developed coating were evaluated. The antimicrobial testing of the GPE showed that the minimum inhibitory concentration (MIC) against E. coli and S aureus was 200mg/ml. For the preparation of edible coating, the MIC was used. The addition of GPE increased the lightness of the coating and made it more yellow. The GPE supplementation increased the opacity of the coating, lowered transparency and protects food against lipid oxidation. The water solubility was higher for the supplemented coating. Higher water solubility indicates that the coating will resist more time in moist conditions. The addition of GPE imparted antioxidant activity, and the IC50 value of the total phenolic content of the coating was 53.61 µg GAE/ mg of coating. The addition of GPE imparted antimicrobial properties. The result of the disc diffusion test showed a zone of inhibition of supplemented edible film was 1mm against E. coli and S aureus. The GPE-supplemented edible coating showed good antioxidants and moderate antimicrobial activity.

Keywords: Edible coating, Antioxidant, Antimicrobial, Food oxidation, Natural additives.

Introduction

Edible coatings/film (EC/EF) is a thin layer of a physical component that can be eaten and provides an effective barrier against oxygen, external microbes, moisture and the chemical/physical changes of the foods [1]. EC used in the food system is mostly a thick solution of components that is coated in the outer layer of foods. The edible layer covers the food product like an envelope. EF/EC are majorly applied in highly perishable food such as horticultural products, meat products etc. [2]. The antimicrobial functionality is added to EC by incorporating antimicrobial agents in the coating solution.

The potential antimicrobial agents used are organic acids (sorbic, benzoic, lactic, propionic, sorbic, acetic); fatty acid esters (glyceryl monolaurate); polypeptides (lysozyme, peroxidase, lactoferrin, nisin); plant essential oils (cinnamon, lemongrass, oregano); and nitrites and sulphites. However, the natural plant extract and essentials oils are believed as a good replacement for the synthetic additives as these natural agents are generally regarded as safe (GRAS) [3]. Starch-based EC /EF are gaining interest amongst the researchers for the development of newer technologies in food packaging such as the incorporation of various active substances in the film-forming solution. The active substances added are mostly antioxidant and antimicrobial agents, which are added to make the edible film more effective against spoilage [4]. Other substances usually added include flavourings, pigment, nutrient, anti-browning compounds, texture enhancers, and probiotics which enhances the functionality of the food [5].

The use of natural additives in the food packaging material has increased in recent days; they are rich in bioactive substances such as antioxidants and antimicrobial agents which the researchers are more interested to work in this field [4]. Essential oils have gained popularity in the food industry as a natural antioxidant and antimicrobial agent in food packaging [6]. A study showed that starch-based edible film supplemented with pomegranate peel powder significantly improved antibacterial

properties against S. aureus and Salmonella of the film [4]. Ginger peel is an inedible part of the rhizome and is considered a waste all over the world. Only ginger pulp is used in food and medicine. Past studies show the potential functionalities of ginger. A study reported that ginger peels have higher total phenols and total flavonoids than ginger pulp [7]. The ginger peels have a good amount of antioxidant and antimicrobial properties and can be utilized as an additive in biofilm [8]. However, utilization of the ginger peel by using its functionality in a product has not been done. With this background, the current study was planned to formulate a ginger peel extract-based edible coating.

Material and Methods

Proximate Analysis of ginger peel: The fresh rhizomes of ginger were procured from the commercial market and cleaned thoroughly. The ginger peels were separated from rhizomes. The moisture content, total ash content, crude fat content, crude fibre content, and protein and carbohydrate content was determined for the ginger peel.

Moisture content: The fresh peels were used for the determination of moisture content by the AOAC (2000) method [9]. The moisture content was calculated using the formula: $(W_1\text{-}W_2/W\text{-}W_1) \times 100$. Where W = Weight in grams, of the petri dish; W_1 = Weight in grams, of petri dish + sample before drying; W_2 = Weight in grams, of petri dish + dried sample.

Ash content: The total ash content of the sample was determined according to the A.O.A.C (2000) official method [9]. The total ash content was calculated using the formula: $(W_2\text{-}W_1/W_1\text{-}W) \times 100$. Where; W = weight in grams of empty dish; W_1 = weight in gram of dish + sample; W_2 = weight in gram of dish + total ash.

Fat content: The crude fat content of the sample was determined by the A.O.A.C (1995) method using petroleum ether as solvent [10]. Crude fat was calculated by using the formula: $(W_1\text{-}W_2)/ S \times 100$. Where W_1 = weight of round bottom flask before extraction; W_1= weight of round bottom flask after the extraction; S = weight of the sample.

Protein content: The protein content was determined by Lowry's method [11]. The absorbance of the samples was determined at 660nm. The standard calibration curve was plotted using bovine serum albumin.

Carbohydrate content: Total carbohydrate content was determined by the difference method. It was calculated from following formula: % carbohydrate = 100- (% moisture + % of ash + % protein + %fat).

Crude fibre content: Determination of crude fibre content was done by the acid-alkali method (AOAC 2000) [9]. A defatted sample was taken for the determination of the ginger peel crude fibre content. The crude fibre content was obtained using the formula: [100- (Moisture + Fat) × weight of fibre]/ Weight of sample taken (defatted).

Preparation of ginger peel extract: The samples of ginger peel were extracted with two solvents. The first extract was prepared by the ratio of 5g sample to 200 ml of 70% ethanol and the second sample was prepared in 70% methanol. The methods of extraction were adopted from Serrano-Díaz et al. with slight modification [12]. Dried powder sample (5 g) was mixed with 200ml of extraction solvent (methanol: water/ ethanol: water) and shaken in an incubator shaker maintained at 40°C and 180 rpm for 12 hrs. The samples were then centrifuged and the supernatant collected. The residue was once again extracted with 200 ml of extraction solvent. The samples were centrifuged and supernatant collected and combined with the supernatant obtained in the first step. The extract was then concentrated under vacuum in a rotary evaporator at 45°C followed by freeze-drying of samples to obtain crude extract in powder form. The extract was transferred in amber coloured airtight containers.

Antioxidant activity of ginger peel extract: The antioxidant activities of both ethanolic and methanolic extracts were evaluated by total phenolic content (TPC), total flavonoid content (TFC), and ferric reducing antioxidant power (FRAP) assay.

Total Phenolic content: The TPC of the samples was determined by a method using Folin–Ciocalteu (F–C) reagent [13]. The intensity of coloured complexes was determined using a UV-visible spectrophotometer (Agilent, USA, Model: Cary 100) at 765 nm. The phenolic content of the samples was expressed as µg gallic acid equivalent (GAE)/g of extract. To obtain a gallic acid calibration curve, the various concentration of gallic acid solutions (0.06µg/ml - 2 µg/ml) was prepared.

Total flavonoid content: The TFC was determined using the method described by Xu and Chang with modifications [14]. The absorbance of the samples was estimated using a UV-visible spectrophotometer (Agilent, USA, Model: Cary 100) at 510 nm.

The total flavonoid content of the samples was expressed as µg quercetin equivalent (QE)/g of extract. To obtain a quercetin calibration curve, the various concentration of quercetin in DMSO (7 µg/ml – 1000 µg/ml) was prepared.

Ferric reducing antioxidant power assay: The FRAP assay was performed as per the method described by Benzie and Strain [15]. The FRAP assay was carried out using a UV-visible spectrophotometer (Agilent, USA, Model: Cary 100) at 593 nm. To obtain a ferrous sulphate calibration curve, the various concentration of ferrous sulphate (2.5mM – 15mM) were prepared. The results were expressed as mM ferrous sulphate equivalent (FSE)/ g of extract.

Antimicrobial analysis of ginger peel extract: Before determining of antimicrobial activity of the extract, the microbial load of the extract was determined. For determination of microbial load 1mg/ml 70% ethanolic extract was used. The microbial load was determined using nutrient agar, eosin methylene blue agar and sabouraud dextrose agar media. 100µl of the extract was spread over the surface of the media and incubated. At the end of incubation time, the plates were examined for microbial growth.

The antimicrobial activity of the 70% ethanolic extract was determined by minimum inhibitory concentration (MIC). The MIC of the extract was evaluated against E. coli and S. aureus. MIC was expressed as mg/ml. The MIC of ethanolic extract obtained was used for the preparation of edible coating.

Development of edible coating: The edible coating was developed by utilizing the ginger peel ethanolic extract as ethanol is considered a GRAS additive. The method for developing starch-based edible coating was as described by Ali et al. with some modifications [4]. The ethanolic extract of ginger peel was used as an antioxidant and antimicrobial agent. Ethanolic extract of ginger peel at a concentration of 200mg/ml was added at 5% (v/v) of the total starch film-forming solution. For forming an edible coating 5g of cornstarch was mixed with 100ml distilled water.

The mixture was heated with stirring until gelatinization. It was then cooled to 25°C and then 30% (v/w) of glycerol was added followed by stirring for 10 min. After this 5% of 200mg/ml (MIC of ethanolic extract), the ethanolic extract was added and mixed followed by pouring. A control edible coating was prepared following the same scheme except for the addition of ginger peel extract.

Physical properties of the edible coating

Water solubility: Water solubility is an indicator of moisture sensitivity. The water solubility of the films was assessed by the method given by Adilah et al. [16]. The film specimens (ginger peel extract-based edible film and control film) were cut into 5 ×5cm dimensions and dried in the oven at 60°C for 24 hours and the initial dry weight was recorded. The dry films were immersed in 3 falcon tubes containing 30ml of distilled water each and were shaken for 1 hour at 200rpm and the temperature was maintained at 30°C. Then the insoluble film was filtered by using filter paper and recorded the weight of the insoluble specimen. It then was transferred to a hot air oven for drying at 60°C for 24 hours. Then the final dry weight of the film specimen was recorded. The water solubility of the films was calculated using the following formula:

Water solubility (%) = initial weight – final weight / initial weight ×100

Colour estimation: The colour of edible coating samples was measured using a colourimeter (Chroma meter CR-400). The L, a*, and b* values which indicate the films lightness, greenness/redness and blueness/yellowness were recorded and* indicates the total colour difference.

Opacity estimation: The opacity of the film was determined according to the method described by Siripatrawan and Harte [17]. The films were cut according to the size of the cuvette cell and were directly inserted. Absorbance was taken at 600nm using a UV-visible spectrophotometer (Agilent, USA, Model: Cary 100). The opacity of the film was calculated by T= Abs 600/x. Where T is the transparency of films, Abs600 is the value of absorbance at 600nm and x is the thickness of films. The higher T values indicate the lower opacity.

Coating thickness: The thickness was measured using a vernier calliper, the thickness was measured in three different areas of the film and the average was taken as a result.

Total phenolic content of the edible coating: The TPC of the edible coating was determined according to the method of Xu et al. with some modifications [18]. Small pieces (2.54*2.54 cm) of the coating were placed into 10 ml absolute ethanol and were shaken for 24 hours at room temperature and further centrifuged at 3500 rpm for 15 minutes. The supernatant was collected for the TPC analysis. TPC of the film was expressed as µg GAE / mg edible coating (µg GAE/ mg) on a dry weight basis.

Antimicrobial activity of edible coating: The disc diffusion method was used to determine the antimicrobial activity of the edible coating. Two different bacterial inoculums (S. aureus and E. coli) were prepared in 10 ml of nutrient broth and incubated for 24 hours at 37°C. Then bacterial cell count was fixed at 106 CFU/ml by recording O.D. at 600nm wavelength. Then suspended colonies of standardized concentration of dilutions were swabbed over the surface of nutrient agar plates using a cotton swab [19]. Along with that 6-8mm diameter ginger peel extract-based edible coating discs were placed on the surface of the agar plate

and ethanol was used as a negative control. Incubation was done at 37°C for 18-24 hours. Ampicillin and neomycin were used as standard.

Statistical Analysis: The data is presented as mean ± SD. All the antioxidant assays of crude extract and proximate analysis of the ginger peel were carried out in triplicate. For estimation of the antioxidant activity of the edible coating, the sample size was five.

Results

Proximate Analysis of ginger peel: The moisture content of the fresh ginger peels was recorded as 88.04 ±0.45% and ash content was 12.83 ± 1.53% (Table 1). The protein content was 18.80± 4.08% and fat content was 3.53 ± 1.00% and fiber content was 21.05 ±4.40%. The carbohydrate content in the ginger peel was found to be 52.56%.

Table 1: Proximate composition of ginger peel

Material	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude Fiber (%)	Carbohydrate (%)
Ginger peel	88.04± 0.45	12.83± 1.53	3.53± 1.00	18.80 ± 4.08	21.05± 4.40	52.56

(Data presented as mean± SD)

Antioxidant activity of ginger peel extract: The comparison of the ethanolic and methanolic extracts showed that TPC content was higher in the methanolic extract as compared to the ethanolic extract as estimated by IC_{50} value (Table 2).

Table 2: Antioxidant activity of ginger peel extract and edible coating

S. No	Sample	Assay	IC_{50} value
1	Ginger peel ethanolic extract	TPC	29.64 µg GAE/g of extract
2	Ginger peel methanolic extract	TPC	14.37 µg GAE/g of extract
3	Ginger peel ethanolic extract	TFC	21.45 µg QE/g of extract
4	Ginger peel methanolic extract	TFC	696.95µg QE/g of extract
5	Ginger peel ethanolic extract	FRAP	8.26 mM FSE/ g of extract
6	Ginger peel methanolic extract	FRAP	7.36 mM FSE/ g of extract
7	Ginger peel extract-based edible coating	TPC	53.61 µg GAE/ mg of coating

However, the results of TFC assay showed that flavonoid content was higher in the ethanolic extract as compared to methanolic extract.

FRAP assay showed that IC_{50} of ethanolic extract and methanolic extract were comparable.

Antimicrobial analysis of ginger peel extract: To check microbial contamination in ginger peel extract three types of media were prepared i.e., eosin methylene blue media (a differential media especially used for isolation of gram –ve bacteria), nutrient agar media (is a non-selective media) and sabouraud dextrose agar media (a selective medium used for yeast isolation). No visible microbial growth was observed in ginger peel extract extracts (fig.1).

Figure 1: No visible microbial growth observed in ethanolic extract of ginger peel

Table 3: Microbial growth examination of ginger peel extract

Sample	Concentration (mg/ml)	Growth media	Incubation time (h)	Incubation temperature (°C)	Microbial growth
Ginger Peel Ethanolic Extract	1mg/ml	NA	18-24	37	No
Ginger Peel Ethanolic Extract	1mg/ml	SDA	18-24	20	No
Ginger Peel Ethanolic Extract	1mg/ml	EMB	18-24	37	No

(NA: nutrient agar media; SDA: sabouraud dextrose agar media; EMB: eosin methylene blue media)

MIC is the lower most concentration of antimicrobial agents that completely inhibit the growth of microorganisms. 200mg/ml concentration was estimated as a minimal inhibitory concentration of ginger peel extract against E. coli and S. aureus (fig.2).

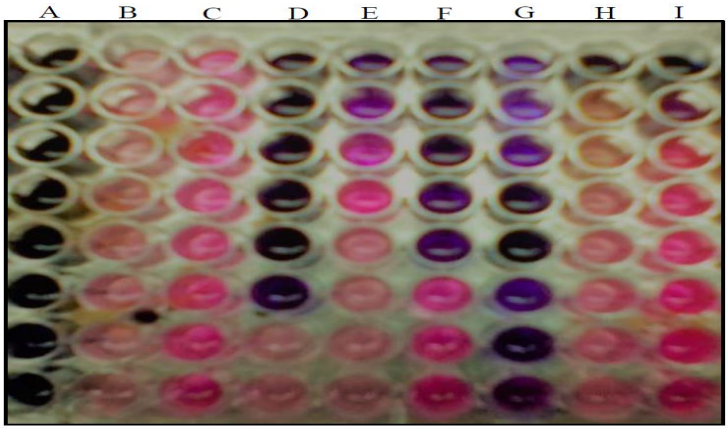

Figure 2: Minimum inhibitory concentration of ginger peel extract (A: sterility control; B: –ve control for E-coli; C: –ve control for S. aureus; D: +ve control (neomycin) for E-coli; E: +ve control (neomycin) for S. aureus; F: +ve control(ampicillin) for E-coli; G: +ve control (ampicillin) for S. aureus; H: ginger peel ethanolic extract for E-coli; I ginger peel ethanolic extract for S. aureus)

Resazurin dye (blue coloured redox indicator) was oxidized by microorganisms to a pink coloured compound resorufin and can be further reduced to hydroresofurin. The intensity of the pink colour indicates antimicrobial activity.

Physical properties of ginger peel based edible coating:

Edible coating colour is one of the factors in terms of consumer acceptance of a product.

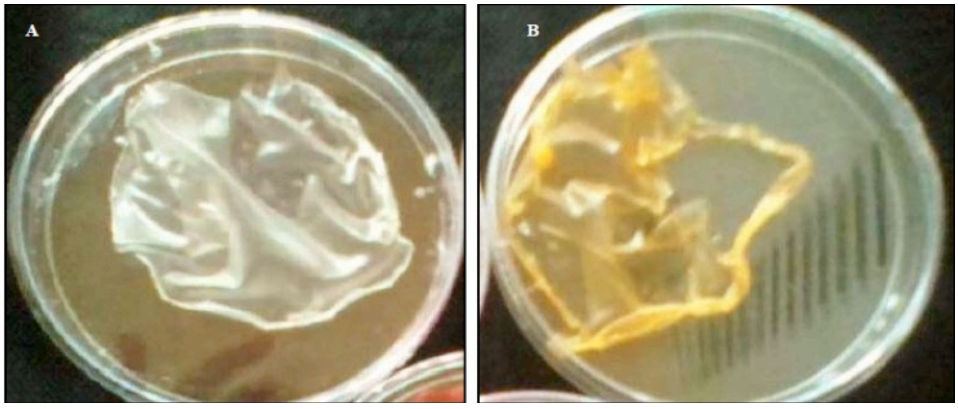

Figure 3: Representative picture of edible coating (A: control coating; B: ginger peel extract-based coating)

The parameter ΔE indicates the degree of total colour difference, a (red-green), b (yellow-blue), L indicates the lightness of coating and Δ signifies the total significant difference. As shown in table 4 the incorporation of ginger peel extract showed a significant difference ($p \leq 0.05$) in the L-value.

Table 4: Colour parameters of ginger peel extract-based edible film

Colour parameters	Control edible coating	Extract based edible coating
L*	39.39±0.56	44.01±0.56
a*	-0.50±0.49	-1.15±0.49
b*	-0.12±0.03	1.57±0.035
ΔL*	-55.19±0.56	-54.76±0.56
Δa	4.55±0.028	4.23±0.023
Δb	-9.26±0.042	-7.56±0.042
ΔE	56.15±0.55	55.44±0.55

(Data presented as total difference Δ and * shows the significant difference)

The ginger peel extract coating was lighter than the control coating.

The a-value of the ginger peel extract-based edible coating was lower than the value of the control edible coating indicating less red than the control coating and the b-value of the ginger peel extract-based edible coating was higher than the control edible coating indicating more yellow than the control coating. Water solubility is the measure of the amount of a substance that can dissolve in water at a specific temperature.

The water solubility of control edible coating was estimated at 28.45 % and 33.69% of ginger peel extract-based edible coating.

Table 5: Water solubility of edible coating

Sample	Initial dry weight (g)	Final dry weight (g)	Water solubility (%)
Control	0.266	0.1903	28.45
Ginger extract-based film	0.119	0.0789	33.69

(Data represented as initial and final weight of coating specimen in grams and water solubility in percentage.)

The results of opacity analysis showed that the T value of the control starch edible coating was estimated to be1.26± 0.5 and the T value of ginger peel extract-based edible coating was estimated to be 7.8± 2.8. The higher T value (transparency value) indicates higher opacity. The addition of ginger peel extract in the film-forming solution significantly increased the opaqueness.

The thickness of the edible coating is an important physical property, which is influenced by the drying temperature, time and method of coating preparation. An increase in the thickness of the coating is because of the gelatinization of starch granules. The mean thickness was measured as 0.1mm for both ginger peel-based coating and control coating.

Total phenolic content of the edible coating: The total phenolic content of the edible coating was determined as mg GAE/mg of film. Ginger peel extract of concentration 200mg/ml was added to make the coating. The TPC of the edible coating was observed as 328.725 ± 0.009 µg GAE/ mg of coating

Antimicrobial activity of edible coating: Zone inhibition is a well-known method to determine the antimicrobial activity of a substance qualitatively. In this method plates were incubated with a standardized inoculum of the test microorganism and 6mm discs containing antimicrobial agents were placed over the surface of the agar plate and incubated under suitable conditions. Antimicrobial agents diffuse in the agar and then the diameter of the inhibition zone was measured. The visible zone of inhibition of ginger peel extract-based film was measured as 1mm against E. coli, and 1mm against S. aureus.

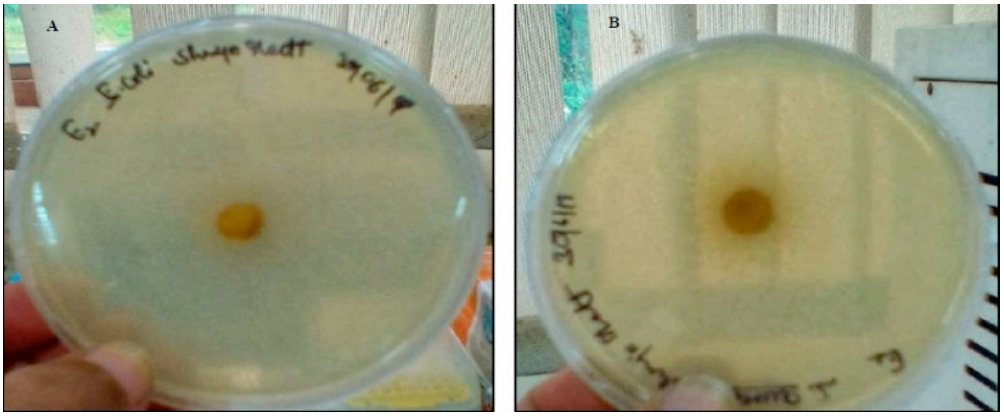

Figure 4: Zone of inhibition of ginger peel extract against E Coli (A) and S. aureus (B).

Discussion

The starch-based edible coating was developed successfully with the addition of 70% ethanolic extract of ginger peel. The physical properties of the coating developed were within the acceptable limits. The results of the colour analysis showed that the addition of the extract into the coating solution increased the lightness of the coating and the coating was more yellow in comparison to the control coating. The results of opacity analysis showed a higher T value of the ginger peel based edible coating than the control starch-based edible coating which indicates lower transparency.

Lower transparency is beneficial in edible packaging as it will protect the food from light i.e., protect from lipid autooxidation [18]. The thickness of the coating was similar to the control coating which is within the acceptable range [20]. Water solubility is the measure of the amount of a substance that can dissolve in water at a specific temperature. The percentage of water solubility of ginger peel extract-based edible coating was higher than the control coating which indicates that the coating will resist more time in moist conditions.

The results of the current study show that ginger peel is a rich source of antioxidants. The ethanolic and methanolic extracts of ginger peel were compared. The extracts were analyzed by different antioxidant assays like ferric reducing power, total phenolic and total flavonoid contents. The phenolic compounds act as primary antioxidants and are mainly free radical scavengers. Phenolic compounds delay the initiation and propagation of chain reactions leading to free radical generation [21]. The ginger peel showed that they are rich in phenolic content as depicted by the TPC assay. The IC_{50} values of the TPC assay were lower for methanolic extract in comparison to ethanolic extract. Indicating the higher antioxidant potential of methanolic extract.

Flavonoids are one of the predominant classes of natural phenolics that account for nearly two-thirds of dietary phenols [22]. The TFC content of the ginger peel extracts (ethanolic and methanolic) showed that the peels are rich in total flavonoids. In the case of TFC, higher flavonoid content was observed for ethanolic extract as it had a lower IC_{50} value. Studies are available that show that ethanol is a superior solvent in comparison to methanol for flavonoid extraction [23].

The results of FRAP assays showed that both methanolic extract and ethanolic extract had similar IC_{50} values. Taken together the results of the antioxidant assay showed that the ginger peel is a rich source of antioxidants and should not be considered a waste, the extraction of these valuable components and utilization into a suitable product could be beneficial to mankind as waste reduction is always profitable. These findings are in line with the literature reports showing that peeled ginger rhizome lacks many essential constituents like shogal, and zingerone [8]. Hence, these findings showed that ginger peel extract is a good material for incorporation into the edible coating for enhancing the antioxidant properties of the edible coating. The addition of ginger peel extract imparted antioxidant activity to the edible coating as evident from the TPC assay of the film. The TPC IC_{50} value of the film indicated a significant antioxidant activity of the film. The antioxidant properties of the coating will protect the food against oxidation.

The results of the antimicrobial assays showed that the ginger peel extract showed activity against microorganisms E. coli and S. aureus although the efficacy was less as compared to standard antimicrobial agents. No visible microbial growth was observed on the surface of the media on which 100µl of ginger peel extract was added. Minimal inhibitory concentration is the lowermost concentration of antimicrobial agents that completely inhibit the growth of microorganisms. 200mg/ml concentration was estimated as the minimal inhibitory concentration of the ethanolic extracts of the ginger peel against E. coli and S. aureus. Zone inhibition is a well-known method to

determine the antimicrobial activity of a substance qualitatively.

The results showed moderate inhibitory activity of the ginger peel based edible coating. As seen in antioxidant activity it may be possible that methanolic ginger peel extract has higher antimicrobial properties, but as the present work aimed at the development of the edible coating so, ethanol was selected as a solvent for extraction as it is safe for human consumption [24].

In conclusion the results of the present study that ginger peel extract has good antioxidant properties and can be used as an antioxidant agent in edible coatings. Moderate antimicrobial properties of the ginger peel extract against microorganisms E. coli and S. aureus were observed. Therefore, it can be concluded that a combination of ginger peel extract with other natural antimicrobial agents can be done for increasing the antimicrobial efficiency of the edible coating.

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