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Research Article

Assessment of Antioxidant and Antimicrobial Property of Polyphenol-Rich Chitosan-Pineapple Peel Film

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This work aimed to evaluate the antioxidant and antimicrobial capacities of pineapple peel extract-incorporated chitosan films to establish its utility as an active food packaging film. Total phenol and total flavonoids in ethanolic pineapple peel extract (11.1 ± 0.82 mg GAE/g sample, 3.86 ± 0.4 mg Quercetin/g sample) were determined to be higher than those in methanolic pineapple peel extract (7.98 ± 0.55 mg GAE/g sample, 2.37 ± 0.13 mg quercetin/g sample) and higher antioxidant activity was observed for pineapple peel ethanolic extract (PEE). Similarly, PEE-enriched chitosan film also reported greater antioxidant activity compared to pineapple peel methanolic extract (PME)-incorporated chitosan film. The total phenols, flavonoids, and significant antioxidant activity were accounted due to the contents of ferulic acids, quercetin, and kaempferol in both PEE and PME quantified via triple quadrupole LC/MS/MS system. These alcoholic extracts exhibited significant inhibitory zones against both Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Salmonella typhimurium*) food-borne bacterial strains. PME exhibited the lowest minimum inhibitory concentration and minimum bactericidal concentration (0.625 mg/ml) against *B. cereus*. Pure chitosan films at ≥7 log CFU/ml after 24 h showed lower log reduction for all the bacterial organisms, whereas the chitosan-PEE (at ≤5 logs CFU/ml) and chitosan-PME (at ≤6 log CFU/ml) films expressed higher log reduction for all the four bacterial isolates. Thus, this work led to the utilization of the pineapple peel waste as well as provided an alternative to nonbiodegradable packaging films.

1. Introduction

Biodegradable packaging materials are considered as one of the top priorities in the food industry due to the increased need for alternative packaging materials that are recyclable, easily degradable, renewable, and require minimal disposal [1, 2]. Chitosan has been commonly used for the development of packaging film due to its inherent film-forming properties along with nontoxicity, biocompatibility or biodegradability properties, chemical reactivity, and high stability [3–5]. The antioxidant property of chitosan was enhanced by incorporating *Thymus moroderi* or *Thymus piperella* essential oils into the chitosan matrix, as chitosan itself does not possess any significant antioxidant activity [6]. Chitosan in solution exhibited an antimicrobial effect due to the surface interaction between the biopolymer chains and the cell wall of the microbes but when chitosan constitutes the matrix for film, it failed to display the inhibitory effect on microbes [7]. With this ambiguous antimicrobial activity and no significant antioxidant activity of chitosan films, researchers have focused their attention on the natural bioadditives for the enhancement of the antimicrobial and antioxidant capacity of chitosan films.

There is extensive and exhaustive usage of fruit peels in the development of chitosan films [8–11]. The incorporation of pomegranate peel extract along with carvacrol increased the antioxidant and antimicrobial properties of chitosan films [8]. Chitosan films cross-linked with banana peel waste

reduced the hydrophilicity and caused a decline in the moisture content, water vapor permeability, and water solubility of the chitosan films. This composite film of chitosan–banana peel extract showed promising antioxidant properties in food simulants and increased the postharvest quality of the apple compared to the pure chitosan films [9]. The incorporation of mango peel extract into chitosan for the development of active packaging enhanced the antioxidant property of chitosan film [10]. The incorporation of polyphenols from apple peel into the chitosan matrix enhanced the antimicrobial and antioxidant capacity of chitosan films [11].

As far as pineapple is concerned, pineapple peel contributes about 29–40% of total pineapple waste [12] and is rich in gallic acid, catechins, epicatechins, ferulic acids, and these bioactive compounds can be utilized as active antioxidant ingredients [13–15]. Constituents such as flavonoids, saponins, and tannins are known to act as natural antimicrobials in reducing the spoilage of food [16–19]. Recently, pineapple has been utilized in the development of food packaging films where pectin from pineapple peel extracted by microwave-assisted technique exhibited plasticizing property for the films in addition to antioxidant properties [20]. Alginate films encapsulating bioactive compounds from pineapple peel showed effective microbial inhibition on meat and also retarded the lipid oxidation of meat [21]. Although pineapple peel has such a rich source of bioactive compounds, this fruit peel has not been yet explored with chitosan for development of active packaging film.

Therefore, in this work, a detailed study to assess the functional properties of chitosan films incorporated with pineapple peel extract (PPE) has been undertaken. In addition, an estimation of total phenol and flavonoid content were measured to address the significant antioxidant capacity of the pineapple peel extracts determined in this work. To account for the major bioactive components in PPE responsible for high antioxidant activity, the methanol and ethanol pineapple peel extracts were characterized and quantified through LC-MS/MS. Further, the antimicrobial activity of the pineapple peel extract was also evaluated in detail as the previous literature was found to be scarce and haphazard.

2. Materials and Methods

2.1. Materials. Pineapple peels were collected from Fresh Grove Agro Pvt Ltd, Sonepat, Haryana, India. Chitosan (medium molecular weight and >75% de-acetylation degree, Cas No. 448869), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2′-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, catechin, epicatechin, ferulic acid, quercetin, and kaempferol (HPLC analytical grade), for chromatographic analysis were purchased from Sigma-Aldrich, Mumbai, Maharashtra, India. Tryptose soy broth, agar-agar, solvents: ethanol and methanol (>99%, HPLC grade), acetic acid, glycerol, Folin–Ciocalteau reagent, aluminum chloride, potassium acetate, copper (II) chloride solution, neocuproine, sodium hypochlorite were purchased from Hi-Media, Mumbai, Maharashtra, India. Resazurin extra pure AR grade and tetracycline hydrochloride, Whatman filter paper No 1, sodium carbonate, potassium persulfate were procured from Sisco Research Laboratory (SRL) Pvt. Ltd., Gurugram, Haryana, India. Two Gram-negative bacterial organisms: *Escherichia coli* (ATCC 5922), *Salmonella typhimurium* (MTCC 98) and two Gram-positive bacterial organisms: *Staphylococcus aureus* (NCDC 109), *Bacillus cereus* (NCDC 240) were procured from Microbial Type Culture Collection (MTCC) & Gene Bank, CSIR-IMTECH, Chandigarh and National Collection of Dairy Cultures (NCDC), Haryana, India, respectively.

2.2. Extraction of Pineapple Peel. Pineapple peel extracts were prepared according to the method [22] with slight modifications. Briefly, freeze-dried pineapple peel powder was dissolved in 100 ml of absolute methanol and ethanol separately in conical flasks in the ratio of 1 : 10. Flasks containing extracts were covered with cotton plugs and boiled continuously for 30 min. The extracts were placed in an orbital shaker (Labilne, Mumbai, Maharashtra, India) for 24 h at ambient temperature (25°C). The extracts were filtered with muslin cloth and again filtered twice by Whatman filter paper No. 1. Extracts were evaporated in a rotary vacuum evaporator (EYELA OSB2100, China) at a temperature between 35°C and 40°C upto dryness and stored at −20°C.

The yield was calculated by using the following equation:

$$\text{extraction recovery}\,(\%) = \frac{\text{weight of recovered dry extract}\ (\text{g})}{\text{intial dry weight of peel powder}} * 100. \quad (1)$$

2.3. Total Phenolic Content (TPC). Total phenolic content present in the pineapple peel ethanolic extract (PEE) and pineapple peel methanolic extract (PME) was evaluated by the Folin–Ciocalteau method [23] with modifications. Briefly, 1 ml of PEE and PME was added to 2 ml of Folin–Ciocalteau (FC) reagent (10%) and 4 ml of sodium carbonate (Na_2Co_3-7.5%). The solution was mixed and incubated for 30 min in dark conditions at room temperature. The absorbance of the sample was measured by UV-Spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan) at 765 nm. Total phenol content was measured in mg of Gallic acid equivalent (GAE)/g of the dry weight of the sample.

2.4. Total Flavonoid Content (TFC). The total flavonoid content of PPEs was evaluated by the aluminum chloride method [22, 24] with slight modifications. Briefly, 1 ml of PEE and PME was mixed with 0.2 ml 10% $AlCl_3$, 0.2 ml of 1 M potassium acetate, and distilled water (5.6 ml), and finally incubated for 30 min in dark conditions at room temperature. The absorbance of the sample was measured at 415 nm by UV-Spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). Total flavonoid contents were expressed in mg of quercetin equivalent (QE)/g of extract.

2.5. Antioxidant Activity. Antioxidant activity of both PEE and PME was evaluated by DPPH (1, 1-diphenyl-2-picrylhydrazyl), $ABTS^+$ (2, 2′-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid), and CUPRAC assays. The absorbance values were measured via a UV spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). Antioxidant activity of the extracts was expressed as μmole of Trolox equivalent (TE)/g of the sample weight.

2.5.1. DPPH Radical Scavenging Activity. The DPPH free radical scavenging activity was measured according to the methodology [25]. Briefly, 100 μl of PPEs were mixed with 3.9 ml of 0.1 mM DPPH ethanolic/methanolic solution. The solutions were shaken vigorously in a vortex and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm.

2.5.2. ABTS Radical Scavenging Assay. The ABTS assay was performed as per method [25] with slight modifications. Equal volumes of 7 mM of 2, 2′-azinobis-3-ethylbenzothiazoline 6-sulfonic acid (ABTS) and potassium persulfate (2.45 mM) solution were mixed and incubated for 16 h in the dark at 25°C to generate ABTS•+ radical. Then, the ABTS•+ solution was diluted in ethanol/methanol to give absorbance 0.7 ± 0.02 at 734 nm. The extracts (0.1 mL) were reacted with ABTS•+ solution (1 mL). The solutions were shaken vigorously in a vortex and incubated for 30 min at room temperature in the dark. The absorbance was measured at 734 nm.

2.5.3. Copper(II) Ion Reducing Antioxidant Capacity (CUPRAC) Assay. Copper (II) chloride ($CuCl_2$) solution (1 ml), neocuproine solution (1 ml), and ammonium acetate buffer (1 ml) were added to a test tube. Testing sample (or standard) and H_2O were added to the initial mixture to make the final volume 4.1 ml [25]. After incubation for half an hour, the absorbance at 450 nm against a reagent blank was recorded.

2.6. Characterization and Quantification of Phenolics Using LC/MS/MS System. The characterization of polyphenols was performed on a triple quadrupole LC/MS/MS system (Q Sight 220; PerkinElmer, Boston, MA, USA). Both qualitative and quantitative analysis of methanolic and ethanolic extracts of pineapple peel with an optimum scan range $m/z = 100$–900, negative ionization mode, ion source electrospray ionization (ESI), nebulizer gas 250 Pa, hot surface-induced desolvation (HSID) temperature 250°C, drying gas 80 Pa, and the infusion flow rates were 10 μL/min was performed. Quantitative analyses of six targeted polyphenols, that is, four phenolic acids (gallic acid, catechin, epicatechin, and ferulic acid) and two flavonoids (quercetin and kaempferol) commonly prevalent in pineapple peels were characterized with high accuracy.

2.7. Antimicrobial Activity

2.7.1. Agar Diffusion Method for Antimicrobial Assessment of Pineapple Peel Extract. Tryptose soy agar plates were prepared. Microbial culture suspensions were prepared following 0.5 McFarland turbidity standards. A 100 μl of bacterial cultures were inoculated and spread with a sterile swab. A total of 6 mm wells were prepared with the help of a borer and loaded with 100 μl (UV sterilized) of PEE and PME with two different concentrations (75 mg/ml and 40 mg/ml). The plates were left at 4°C for 30 min for the diffusion of pineapple peel extract and after that, the plates were incubated in a BOD incubator (Forma 4111TS incubator, Thermo Fisher Scientific, Ohio, US) at 37°C for 24 h. A zone of inhibition was observed and measured (in mm) for antibacterial activity. The experiment was done in triplicate.

2.7.2. Minimum Inhibitory Concentration (MIC) for Pineapple Peel Extract. MIC of PPEs was determined by the 96 well titer plate method [26]. A quantity of100 μl of the PPEs (both PEE and PME) was dispensed in each well of column 1 and 50 μl of tryptose soy broth in columns 2 to 10.

The concentrations of PPEs were prepared by serial dilution method ranging from 80 to 0.15 mg/ml. A 50 μl of the bacterial suspension was then added to all the wells making the final volume of each well up to 100 μl. Plates were incubated for 24 h at 37°C with sufficiently moist conditions. A 30 μl solution of 0.015% (w/v) resazurin was added to all the wells and further incubated for 4 to 5 h for the appearance of the color. The blue color showed resazurin and the pink color showed reduced resorufin due to the presence of live bacterial cells indicating the minimum inhibitory concentration of the extracts.

2.7.3. Minimum Bactericidal Concentration (MBC) for Pineapple Peel Extract. The sectoral method was used to assess and quantify the bactericidal effect of the PPEs. Here, each well of MIC assay plates was further subcultured through streaking by using the inoculating loop on sectors prepared in tryptose soy agar plates [27]. The MBC of the extracts was observed after 24 h of incubation at 37°C.

2.8. Preparation of Chitosan Films. The chitosan films were prepared according to the method [28] with slight modifications. Briefly, a chitosan solution of 2% (w/v) was prepared in 1% (v/v) acetic acid. The solution was stirred overnight at room temperature until it got completely dissolved. Glycerol (1.5 ml/g polymer dry matter) was added as a plasticizer and stirred for 30 min. To that solution, PEE and PME were added to reach a final concentration of 1% (w/v) and stirred for 30 min. The film-forming solutions, CH-PEE, CH-PME, pure chitosan (CH) film (as control) were poured on plastic plates and dried for 48 h at 40°C. The dried films were stored in a desiccator (ABDOS E11612, India) at 25°C until further analysis.

2.8.1. Sample Preparation for Antioxidant Activity of Chitosan Films. A 0.1 ml of film solution (obtained by soaking 125 mg chitosan–PPE film in 15 ml ethanol/methanol for 24 h) was used for the analysis of antioxidant activity [29]. Antioxidant activity of both CH-PEE and CH-PME film solutions was evaluated by DPPH, ABTS, and CUPRAC assays.

2.8.2. Antimicrobial Assessment of Chitosan-PPE Films

(1) Agar Disc Diffusion Assay. The agar disc diffusion method was used for the determination of the antibacterial activity of the chitosan films incorporated with pineapple peel extract [30, 31]. Briefly, 100 μl of bacterial cultures (10^5 CFU/ml) were inoculated and spread with a sterile swab. A disk of 6 mm was prepared for both CH-PEE and CH-PME with the help of a borer and placed on the agar Petri plates. The plates were incubated in a BOD incubator at 4°C for 45 min for the diffusion of the extracts and then at 37°C for 24 h. A zone of inhibition was observed and measured for antibacterial activity. The experiment was done in triplicates.

(2) Macrodilution Assay. For the macro dilution assay of the chitosan-PPE films, briefly all the three films (6 mm in diameter) were placed in sterile tubes containing 5 ml of sterile tryptose soy broth. After 15 min of duration, the tubes were inoculated with the 10^5 colony-forming unit (CFU) mL^{-1} of bacterial inoculums and incubated at 37°C for 24 h. A 0.1 ml of an aliquot from these tubes were subcultured on TSA plates, and the plates were incubated for 37°C for 24 h [31]. The results were expressed as CFU/ml compared to the control sample (sterile filter paper disc). All the experiments are done in triplicate and inhibition growth was calculated as log reduction using the following equation:

$$\text{Log reduction} = \text{Log No} - \text{Log } N, \tag{2}$$

where Log No is the initial viable cell count and Log N is the final viable cell count.

2.9. Statistical Analyses. Statistical analyses were carried out using Origin 2019 software to calculate the mean and the standard deviation for extract recovery yield and zone of inhibition (by measuring the diameter of the inhibition zone). Data were expressed as mean ± standard deviation ($n = 3$). Duncan's test was carried out using SPSS (Version 25.0) software to determine the significant differences at $p \leq 0.05$ between the zone of inhibition with different solvents with different concentrations and bacterial species.

3. Results and Discussion

3.1. Extraction Yield. Following the similar process of extraction, the recovery yield from freeze-dried PEE was found to be negligibly higher (10.43% ± 0.51) than the PME (9.08% ± 1.96). This observation was found almost similar to the report [32], where the methanolic extract has an 8.4% recovery, while 7.9% ethanolic extract was obtained from pineapple peel of Maharashtra, India, through the Soxhlet method of extraction. Ramli et al. (2020) showed a 20% recovery yield for the methanolic extract of Malaysian region pineapple peel by the maceration extraction technique [33]. A higher recovery rate (20%) was reported [34] for 98% ethanolic extract of pineapple peel from Nigeria region, where the extraction was done by air drying at room temperature for 72 h. This dissimilarity may be attributed to the different methods of extraction and drying. Further, the geographical origin of plant and the natural environment conditions also plays vital role in composition and constituents of bioactive compounds [35, 36].

3.2. Phenolics and Flavonoids. Polyphenolic compounds are important secondary metabolites that play major roles in biological activities, mostly acting as an antioxidant agent by inactivating lipid-free radical chains, chelating redox-active metal ions, and preventing hydroperoxide conversion into reactive oxyradicals [37, 38]. Total phenol (TPC) and total flavonoid content (TFC) of PPEs and chitosan-PPE film are displayed in Table 1. TPC of PME was determined as 7.98 ± 0.55 mg GAE/g which was found same as the oven-dried methanolic pineapple peel extracted by reflux method [13]. TPC (11.1 ± 0.82 mg GAE/g dry weight) and TFC (3.86 ± 0.4 mg QE/g dry weight) was found to be considerably higher in PEE in the current work compared to the work of Suleria et al. [39], where they reported TPC and TFC as 7.83 ± 0.35 mg GAE/g dry weight and 1.47 ± 0.07 mg QE/g dry weight, respectively, for freeze-dried ethanolic extract of pineapple peel. TFC of PME was determined as 2.37 ± 0.13 mg QE/g dry weight. This is to be noted that after incorporating PPEs into the chitosan matrix, TPC and TFC of the CH-PPE films decreased significantly ($p < 0.05$) compared to the crude pineapple peel extracts. This decrease was probably due to the strong electrostatic interaction between pineapple peel extract and chitosan which regulated the release of polyphenolic compounds from the chitosan film. Although TPC and TFC decreased in the chitosan films compared to the crude extracts, the trend was maintained where CH-PEE film was found to be more enriched with phenols and flavonoids compared to CH-PME film. TPC measured in chitosan solution is probably due to the reaction of Folin–Ciocalteu (FC) reagent with non-phenolic reducing substances which resulted in the formation of chromogens [40] as was detected in the spectrophotometer.

3.3. Qualitative and Quantitative Analysis of Major Polyphenols. LCMS-MS analysis was performed to get a complete qualitative and quantitative estimation of polyphenolic constituents present in the pineapple peel extracts. Figure 1 shows an untargeted qualitative chromatogram of ethanolic and methanolic pineapple peel extracts. Numerous peaks were observed in both ethanolic and methanolic extracts indicating the presence of similar compounds in the extracts. The presence of p-Coumaroyl-caffeoylglycerol ($m/z = 399.1$) and Dicaffeoylglycerol ($m/z = 415.0$) were confirmed in both PEE and PME by comparing m/z values of pineapple peel as reported earlier [41]. According to the previous reports [42] N, N-di-feruloyl spermidine ($m/z = 498.3$) in pineapple peel and pulp, a peak at the same m/z value was observed in both the extracts while peaks for

Table 1: Total phenol content (TPC) and total flavonoid content of (TFC) of PPEs and chitosan-PPE films.

Sample	TPC	TFC
PME	7.98 ± 0.55^{c}	2.37 ± 0.13^{ab}
PEE	11.1 ± 0.82^{d}	3.86 ± 0.4^{c}
CH-PME	4.30 ± 0.2^{b}	1.90 ± 0.02^{a}
CH-PEE	5.14 ± 0.08^{b}	2.59 ± 0.24^{b}
CH	2.51 ± 0.25^{a}	0.09 ± 0.002^{a}

All values are expressed as mg/g means ± standard deviation ($n = 3$). Alphabetic letters indicate the significant difference ($p < 0.05$) within a column using a one-way analysis of variance (ANOVA).

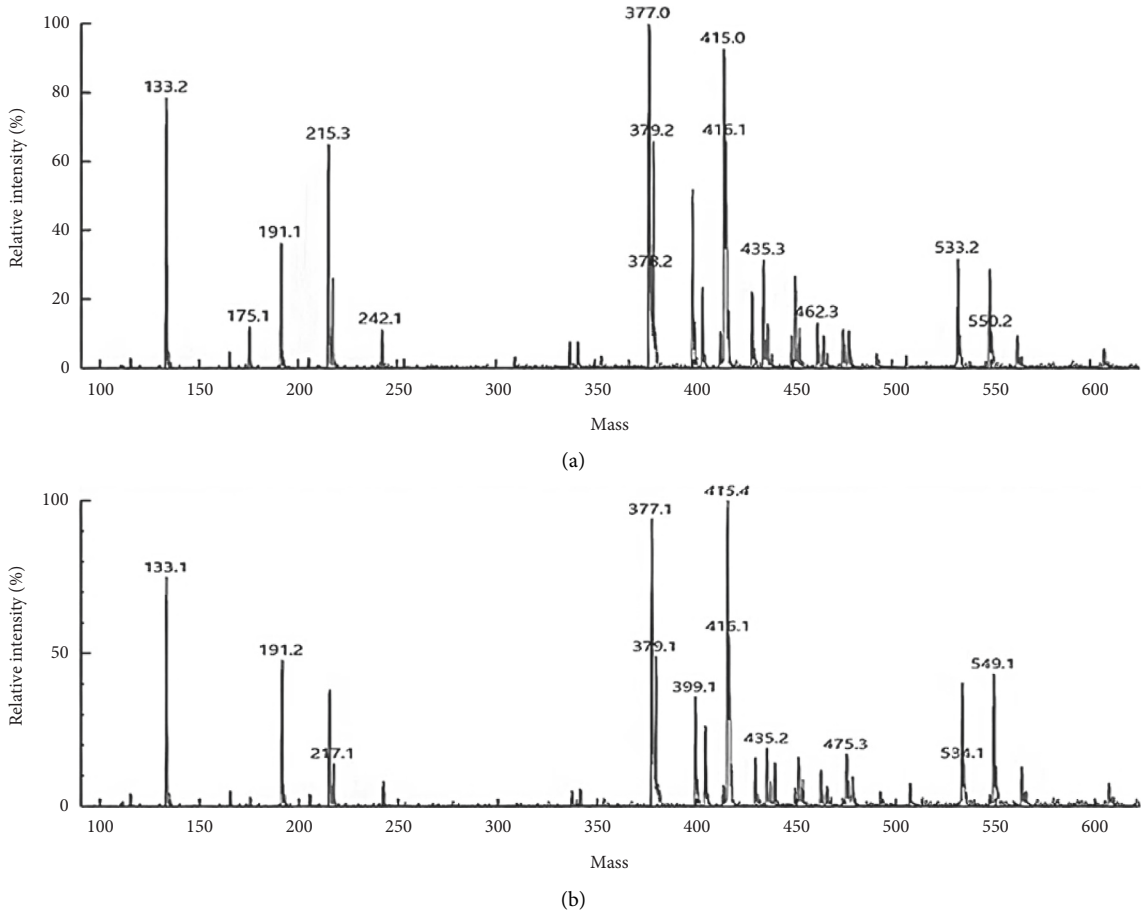

Figure 1: LC-MS-MS chromatogram (negative mode) of PEE (a) and PME (b) ranging from 100 to 600 nm.

serotonin ($m/z = 177.2$) and cinnamic acid ($m/z = 149.3$) were only observed in PME. The presence of kaempferol 3,7-*O*-diglucoside ($m/z = 609.2$) [39] and quinic acid ($m/z = 191.1$) [43] were established in PEE.

Peaks for phenolic acids (gallic acid, ferulic acid, catechin, and epicatechin) and flavonoids (kaemferol and quercetin) were identified by comparing their retention time and pseudomolecular ion $[M\text{-}H]^{-}$ in both ethanolic and methanolic extracts of pineapple peel. A quantitative run of pineapple peel methanolic and ethanolic extracts with the authentic standard was performed and results are presented in Table 2. Both PME and PEE were found to be rich in ferulic acid. PME was found to be higher in gallic acid (332.01 ± 1.91 mg/g dry weight) and ferulic acid (4178 ± 28.85 mg/g dry weight) compared to PEE (gallic acid-165.22 ± 4.49 mg/g dry weight, ferulic acid-3477.6 ± 56.85 mg/g dry weight). On the other hand, catechin was found to be higher in PEE (76.12 mg/g dry weight) than PME (36.98 ± 0.59 mg/g dry weight), whereas epicatechin content was similar in both the extracts (PME-42.428 ± 1.63 mg/g dry weight, PEE-51.95 ± 2.61 mg/g dry weight). In the case of flavonoids, both quercetin and kaempferol were found to be slightly higher in PME (251.84 ± 3.99 mg/g dry weight, 413.26 ± 10.49 mg/g dry weight) compared to PEE (228.12 ± 1.88 mg/g dry weight, 386.74 ± 7.29 mg/g dry weight).

3.4. Antioxidant Activity. The presence of antioxidant capacity in different parts of the fruits is due to the large varieties of bioactive components and measuring the

Table 2: LC-MS-MS quantification of polyphenols in pineapple peel extracts.

Compound	Retention time Min	Molecular weight	$[M-H]^-$ (m/z)	Content (mg/g dry weight) PME	PEE
Gallic acid	2.79	170	169.2	332.01 ± 1.91^{c}	165.22 ± 4.49^{bc}
Catechin	3.66	290	289.1	36.98 ± 0.59^{a}	76.13 ± 1.99^{ab}
Epicatechin	4.20	290	289.2	42.42 ± 1.63^{a}	51.95 ± 2.61^{a}
Ferulic acid	5.25	194	193.3	4178 ± 28.85^{e}	3477.6 ± 56.85^{e}
Quercetin	6.39	302	301.1	251.84 ± 3.99^{b}	228.12 ± 1.88^{a}
Kaempferol	6.92	286	285.3	413.26 ± 10.49^{d}	386.74 ± 7.29^{d}

All values are expressed as mg/g means ± standard deviation ($n = 3$). Alphabetic letters indicate the significant difference ($p < 0.05$) within a column using a one-way analysis of variance (ANOVA).

antioxidant activity of each of the compounds is critical and time-consuming. Therefore, it is necessary to determine the in-vitro antioxidant capacity of plant extracts by combining more than one method [44]. Here in this work, the antioxidant activity of PPEs and the chitosan-PPE film was determined by three different methods: DPPH radical scavenging assay, ABTS, and CUPRAC (Table 3). These different methods for the determination of total antioxidant activity differ in their mode of generating free radicals.

DPPH free radical is organic nitrogen radical, when mixed with antioxidants, turns its purple color to yellow of the corresponding hydrazine. The antioxidant activity (via DPPH assay) was determined as $5.82 \pm 0.28\,\mu$mole TE/g and $13.63 \pm 3.66\,\mu$mole TE/g for PME and PEE respectively, which is much lower than Lourenço et al., where very high DPPH radical scavenging activity (91.79 μmole TE/g dry extract) for ethanolic pineapple peel extract was reported [43]. However, in an earlier study, the antioxidant activity by DPPH assay was found to be quite lower when methanolic pineapple fruit extract (0.89 μmole TE/g) and ethanolic pineapple peel extract (0.78 μmole of TE/g) have been extracted by pulverization method [45]. This difference in the radical scavenging activity might be due to the number of antioxidants present in different parts of the plant, different methodology employed for the extraction process, and also differences in the geographical regions of the plant species. ABTS radical gives bluish-green color at absorbance 743 nm, which was formed by the loss of an electron by the nitrogen atom of ABTS. In the presence of antioxidants, the nitrogen atom quenched the hydrogen atom resulting in the decrease of absorbance with a colorless solution. ABTS assay also estimated higher antioxidant activity in PEE ($49.80 \pm 0.1\,\mu$mole TE/g) than PME ($13.60 \pm 0.50\,\mu$mole TE/g). In the CUPRAC assay, Cu (II) is reduced to Cu (I) by the action of electron-donating antioxidants. Antioxidant capacity determined by CUPRAC assay followed the same trend similar to DPPH and ABTS assays with higher antioxidant activity in ethanolic extract ($6.28 \pm 0.40\,\mu$mole TE/g) compared to PME ($3.45 \pm 4.44\,\mu$mole TE/g). All three assays reported high antioxidant activity of PEE compared to PME (Table 3), and this observation corroborated with higher TPC and TFC for PEE than in PME.

The nature of the high antioxidant activity of PEE is also exhibited in the CH-PEE film. As shown in Table 3, the CH-PEE film showed the highest antioxidant activity followed by CH-PME and the pure CH film showed minimal antioxidant capability as measured by all the three antioxidant assays.

Table 3: Antioxidant activity of chitosan and chitosan-PPE films.

Sample	DPPH	ABTS	CUPRAC
PME	5.82 ± 0.28^{ab}	13.60 ± 0.50^{b}	3.45 ± 0.44^{b}
PEE	13.63 ± 3.66^{c}	49.80 ± 0.10^{d}	6.28 ± 0.40^{c}
CH-PME	3.36 ± 0.28^{ab}	8.031 ± 0.56^{b}	2.48 ± 0.35^{b}
CH-PEE	6.23 ± 1.52^{b}	26.40 ± 0.72^{c}	6.23 ± 0.30^{c}
CH	2.36 ± 0.27^{a}	3.36 ± 0.49^{a}	1.16 ± 0.12^{a}

All values are expressed as mg/g means ± standard deviation ($n = 3$). Alphabetic letters indicate the significant difference ($p < 0.05$) within a column using a one-way analysis of variance (ANOVA).

3.5. Antimicrobial Activity

3.5.1. Determination of Zone of Inhibition. Inhibition zone diameters with PME and PEE at two different concentrations, that is, 75 mg/ml and 40 mg/ml against four different bacteria, are shown in Table 4. It was found that the zone of inhibition increased with an increase in the extract concentration. PEE exhibited a slightly higher zone of inhibition for both Gram-positive and Gram-negative bacterial strains, which are more prominent in *E. coli* and *B. cereus*. The maximum zone of inhibition for PEE (75 mg/ml) against *E. coli* was 21 ± 1.0 mm, while in PME, it was 16 ± 3.4 mm. Sharma and Sharma (2017) reported a 21 mm inhibition zone for PEE and 20 mm for PME against *E. coli* at a 30 mg/ml concentration for both the extracts [22]. Dabesor et al. reported a 12.3 mm zone of inhibition for PEE against *E. coli*. The maximum zone of inhibition for PME and PEE was 16 ± 1.5 mm and 17 ± 1.00 mm, respectively, against *S. typhimurium* in this work [34].

In the case of Gram-positive bacterial organisms, PME and PEE showed a maximum inhibition zone of 12 ± 0.0 mm and 15 ± 1.5 mm, respectively, against *B. cereus*. The pineapple peel extracts did not display any zone of inhibition at 40 mg/ml against *S. aureus* but exhibited 11 ± 1.0 mm as the zone of inhibition for both the extracts at 75 mg/ml. Pineapple peel ethanolic extract (98%) exhibited an inhibition zone of 18 mm against *B. Cereus* [34]. Methanolic and ethanolic extracts of pineapple peel exhibited 15 mm and 14 mm zones of inhibition, respectively, against *S. aureus* at a 30 mg/ml [22].

Table 4: Zone of inhibition of pineapple peel extracts against bacterial organisms.

Bacterial strain	Zone of inhibition (mm)			
	PME		PEE	
	75 mg/ml	40 mg/ml	75 mg/ml	40 mg/ml
E. coli	16 ± 3.4^{b}	14 ± 1.0^{a}	21 ± 1.0^{b}	16 ± 1.7^{b}
S. typhimurium	16 ± 1.5^{b}	10 ± 0.5^{a}	17 ± 1.0^{b}	10 ± 1.5^{a}
B. cereus	12 ± 0.0^{a}	10 ± 1.0^{a}	15 ± 1.5^{b}	11 ± 0.5^{a}
S. aureus	11 ± 1.0^{a}	ND	11 ± 1.5^{a}	ND

All values are expressed as mg/g means ± standard deviation ($n = 3$). Alphabetic letters indicate the significant difference ($p < 0.05$) within a column using a one-way analysis of variance (ANOVA).

Table 5: MIC and MBC of pineapple peel extract against bacterial organisms.

Bacterial strain	MIC (mg/ml)		MBC (mg/ml)	
	PME	PEE	PME	PEE
E. coli	2.5	2.5	2.5	2.5
S. typhimurium	40	40	20	40
B. cereus	0.62	1.25	0.62	1.25
S. aureus	20	20	20	20

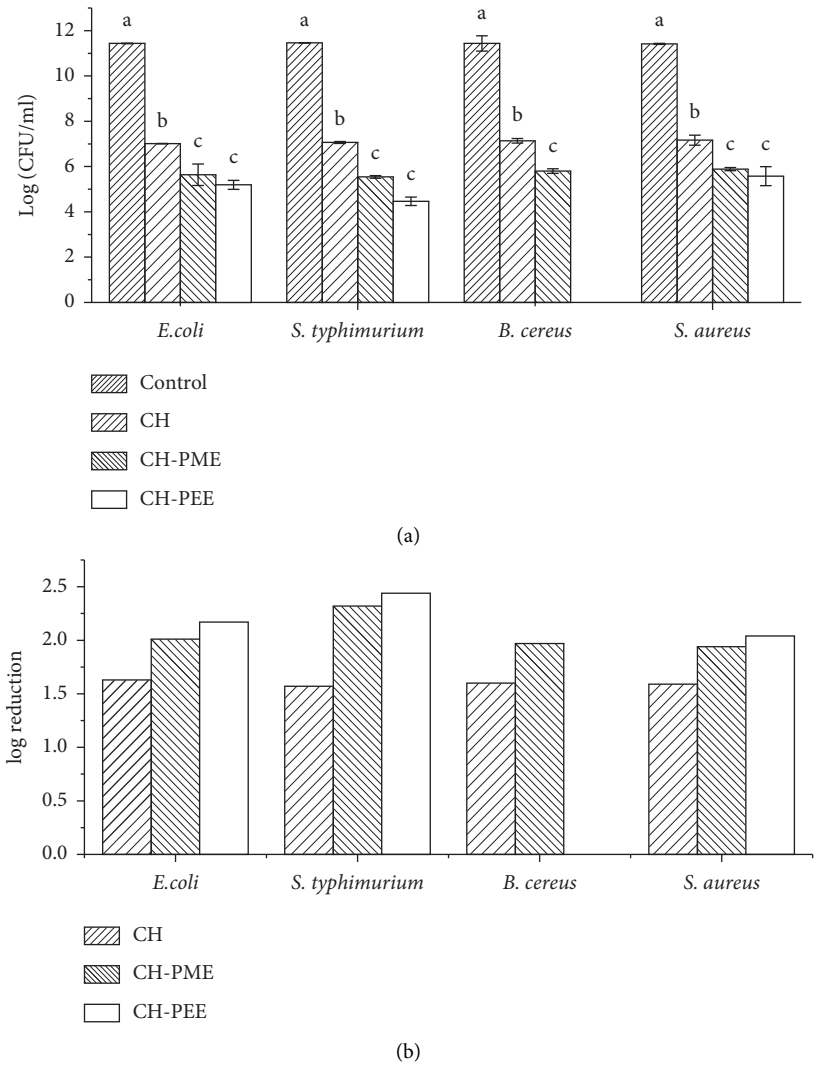

Figure 2: Antibacterial activity of chitosan film incorporated with PME and PEE, where (a) log (CFU/ml) and (b) log reduction value.

The small differences in the different works might be due to the different means of extraction process, the volatile nature of chemical constituents in the extracts, and the bacterial strains used [16, 46].

3.5.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The minimum inhibitory concentration (MIC) is defined as the lowest value of the concentration of an active antimicrobial agent that prevents the visible growth of bacteria (bacteriostatic effect) and is used to evaluate the antimicrobial efficacy of various compounds. On the other hand, minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium (bactericidal effect) [47]. The MIC and MBC values of the pineapple peel extracts were nearly similar against the four pathogenic microorganisms (Table 5). However, MIC and MBC of PME and PEE against *B. cereus* and *E. coli* were much lower than *S. typhimurium* and *S. aureus*. A considerably low value of 0.625 mg/ml was observed for PME against *B. cereus* with MBC at 1.25 mg/ml. The pineapple peel extracts showed the same inhibitory and bactericidal activity except for *S. typhimurium*, where MIC for PME was higher (40 mg/ml) than the MBC (20 mg/ml).

3.6. Antimicrobial Activity of Chitosan-Pineapple Peel Films. The zone of inhibition was not observed for chitosan-pineapple peel extract films. However, there was no growth visible on the surface of films placed on the agar plates. The absence of a zone of inhibition was possibly due to the incapability of chitosan and the antimicrobial compound to diffuse out on the solid agar matrix [7, 48]. The antimicrobial compounds are unable to diffuse out due to the curling nature of chitosan film. Chitosan films curled up on the agar surface, which resulted in a decrease of surface and contact areas between film and culture on an agar plate that led to less diffusion [49].

As per Figure 2(a), significant differences ($p < 0.05$) in log CFU/ml values were observed between pure chitosan (CH) films and CH-PEE, CH-PME films. Pure chitosan films showed ≥7 log CFU/ml after 24 h, whereas chitosan films enriched with methanolic and ethanolic extracts showed ≤6 log CFU/ml and ≤5 log CFU/ml, respectively, after 24 h of incubation in all the four microbial isolates. Pure CH films at 7 log CFU/ml after 24 h showed log reduction of 1.63, 1.6, 1.51, and 1.57 for *E. coli*, *B. cereus*, *S. aureus*, and *S. typhimurium*, respectively. On the other hand, chitosan-PEE and chitosan-PME films showed higher log reduction for all the four bacterial isolates (Figure 2(b)). Thus, the incorporation of PPEs into the chitosan films improved the antibacterial activity of the resultant composite films and supported the findings that chitosan film shows better antimicrobial activity in a liquid state than in the films [7, 48].

4. Conclusion

In this study, chitosan films were successfully developed with pineapple peel extracts. The significant polyphenols of the pineapple peel extracts were identified and quantified through LC-MS/MS which attributed to the antimicrobial and antioxidant properties of the chitosan films. The total phenolic and flavonoids content of ethanolic and methanolic pineapple peel extracts were found to be in correlation with their corresponding antioxidant activities. The antioxidant capacity of the pineapple peel extracts was retained to a considerable extent when incorporated in the chitosan films.

Similarly, these polyphenolic compounds in the crude extracts as well as in the chitosan films demonstrated antibacterial activity when tested against four common food-borne bacterial pathogens. The extracts were found to be more effective against Gram-negative bacterial strains as a higher zone of inhibition was observed against them compared to the Gram-positive bacterial organisms. Minimum inhibitory concentrations and minimum bactericidal concentrations correlated well and conclusively proved that the pineapple peel extracts do have significant antibacterial activity even when they are formulated in a chitosan matrix.

Thus, this work elaborated the process development and functional characterization of pineapple peel-based chitosan film, which also provided an effective eco-friendly utilization of the pineapple peel wastage. Future studies on the physical, mechanical, barrier, and thermal properties will provide information about the viability of this pineapple peel-based chitosan film for practical use in food packaging. This active development of food packaging will enhance the shelf life of food products, which will benefit the consumer in terms of environmental and health issues.

Data Availability

All the relevant data have been provided in the manuscript. The authors will provide additional details if required.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Jyoti contributed to conceptualization, investigation, formal analysis, methodology, validation, visualization, and writing—original draft, review, and editing. Ayon Tarafdar contributed to writing—review and editing. S. Chakkaravarthi contributed to visualization, writing—review and editing, and supervision. B. Bhattacharya contributed to conceptualization, visualization, resources, writing—review and editing, project administration, and supervision.

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