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Antioxidant/antimicrobial potential of *Emblica officinalis* Gaertn and its application as a natural additive for shelf life extension of minced chicken meat

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ABSTRACT

Indian gooseberry (*Emblica officinalis Gaertn.*) is an important fruit used in traditional Indian medicine. Gooseberry was air and freeze dried. The major bioactive components, antioxidant and antibacterial activity of freeze dried (FD) and air dried (AD) gooseberry extracted with three solvents were assessed. Overall, AD gooseberry extracted with 70% ethanol had the highest yield of bioactive compounds. The major phytochemicals identified in the extract by high performance liquid chromatography with UV-visible detector (HPLC-UV-vis) was ascorbic acid, caffeic acid, ellagic acid and ferulic acid. Gram positive bacteria tested were more sensitive to gooseberry extract (GE) as compared to Gram negative organisms. In minced chicken meat, GE was an effective natural food additive as it enhanced its shelf life during chilled storage. GE minimized both bacterial spoilage and oxidative rancidity in chicken. Air dried gooseberry extract (AD-GE) due to its antioxidant and antibacterial activities can be efficiently used as a natural food additive in meat preservation.

Keywords: *Gooseberry; antioxidant; antibacterial; shelf-life extension; chicken.*

1. INTRODUCTION

Consumption of fruits, vegetables, and other plant-based foods has a positive effect on health and prevention of disease. They are a rich source of phytochemicals and a lot of research on their beneficial properties is being carried out. These phytochemicals form the backbone of traditional medicine and have been used effectively since ancient times. *Emblica officinalis* Gaertn (*Phyllanthus emblica*, Indian gooseberry) is one such plant that has been used in traditional Indian medicinal system of Ayurveda. It belongs to family Euphorbiaceae and is one of the important indigenous fruits of the Indian subcontinent. India ranks first in the world in area and production of this crop [1]. In India, it is commonly known as "Amla" and consumed in the form of pickle, juice, candy, chutney, jam etc. Indian gooseberry is the main ingredient in Chawanprash, an ayurvedic jam that is consumed as an herbal tonic having health benefits. Gooseberry is also used in the cosmetic industry for making skin and hair products. It is sour in taste and is attributed to have laxative, hepatoprotective and diuretic properties [2 - 4]. Gooseberry has been reported to have hypolipidemic, hypoglycemic, anti-inflammatory and anticancer [5, 6] properties as well. There are few reports on its antioxidant and antibacterial properties [7, 8].

Food safety has been associated by consumers with attributes such as the absence of harmful organisms and chemicals including synthetic additives. Almost all processed foods contain synthetic food additives. However, these synthetic additives could be toxic and hence consumers prefer natural food additives which in addition to improving the shelf life of food also have other beneficial health effects. Epidemiological studies strongly suggest that long term consumption of plant polyphenols protect against the development of cancer, diabetes, osteoporosis, neurodegenerative, cardiovascular diseases and slow down the aging process. Indian gooseberry is consumed fresh or in processed form. Gooseberry powder is used for making beverages and also for medicinal and cosmetic applications [9]. For these applications drying of gooseberry is an important step that could affect the bioactive properties of the fruit. The main objective of this study hence was to evaluate the effect of drying method and the solvent used on the antioxidant and antibacterial property of gooseberry. Further, the potential of using gooseberry extract as a natural additive for the shelf life extension of minced chicken during chilled storage was also investigated.

2. EXPERIMENTAL SECTION

2.1. Chemicals

Linoleic acid, 1,1-diphenyl-2-picrylhydrazyl, β carotene, nitro blue tetrazolium (NBT), catechin, ascorbic acid, gallic acid, caffeic acid, vanillic acid, chlorogenic acid, ellagic acid, ferulic acid, and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile of HPLC grade was obtained from Merck (Darmstadt, Germany). Other reagents used were of analytical grade and were acquired from HiMedia (Mumbai, India) or Qualigens Fine Chemicals (Mumbai, India).

2.2. Preparation of Gooseberry extract (GE).

Gooseberry was purchased from local market, washed under tap water, pitted and cut into small pieces. It was then divided into two lots. One lot of the gooseberry pieces were dried in a hot air oven (Cintex Precision Hot Air Oven, Mumbai, India) at 40°C for 24 hours and ground into a fine powder. This was the

air-dried (AD) gooseberry powder. The other lot of gooseberry pieces was macerated in a mortar and pestle under frozen condition using liquid nitrogen and then lyophilized. This was assigned as the freeze-dried (FD) gooseberry powder. AD and FD gooseberry powder were extracted using thee solvents - distilled water (D/W), 70% and 100% ethanol incubated at RT (28°C) for 24 h in a shaker incubator (150 rpm). After centrifugation (12,100 x g for 30 min) and filtration (Whatman No. 4), the extract obtained was stored at 0-3°C.

2.3. Total phenolic, flavonoid, tannin and ascorbic acid content of GE.

The total phenolic content of the AD and FD gooseberry extracted using the three solvents were estimated by the method of Singleton and Rossi [10]. Catechin was used as the standard and total phenolic content of GE was expressed as mg catechin equivalent/g. The total amount of flavonoids present in GE was determined using the method of Kim et al. [11]. The absorbance of the pink color developed was read at 510 nm and results were expressed as catechin equivalents. The total tannin content of GE was determined by the protocol of Price et al. [12]. The ascorbic acid content of GE was studied by the method of Albrecht [13].

2.4. Antioxidant activity of GE.

Different in vitro antioxidant assays were carried out to ascertain the antioxidant potential of GE.

2.4.1. DPPH assay. DPPH radical scavenging activity of GE was estimated according to the method of Yamaguchi et al. [14]. Briefly to the appropriately diluted sample DPPH reagent (1 ml) was added, vortexed and incubated in the dark for 20 min. Absorbance was measured at 517 nm and results were expressed as percentage scavenging.

2.4.2. Superoxide scavenging assay. Superoxide radical was generated as described by Liu et al. [15]. A decreased absorbance of the reaction mixture at 560 nm indicated higher superoxide radical scavenging activity.

2.4.3. β carotene assay. β carotene bleaching assay as described by Velioglu et al. [16] was followed for determining the antioxidant activity coefficient (AAC) of GE. An emulsion was prepared using β carotene, linoleic acid, Tween 80 and water. Oxidation was induced by heating the emulsion with/without GE and OD were measured at 470 nm initially and after sixty minutes.

2.4.4. Reducing power. The reducing power of the samples was determined according to the method of Oyaizu [17]. An aliquot of the sample was mixed with of 200 mm sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide and the mixture was incubated at 50°C for 20 min. About 10% Trichloroacetic acid (TCA) was added and the mixture was centrifuged at 650 g for 10 min. The upper layer (5 mL) was mixed with 5 mL of D/W and 1 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm.

2.5. Identification of phenolic compounds present in GE using HPLC.

The individual compounds present in GE were identified by HPLC (JASCO PV2089 Plus, Japan with a KNAUER UV-VIS Detector 2600, Germany). AD and FD GE (1mg/ml) were prepared in methanol and filtered through 0.20 sterile syringe filter (PALL Life Sciences, USA). Injection volume was 20 µl. The column used was ODS Hypersil (Thermo Scientific, USA, 240 mm x 4.6 mm with 5µm pore size). A linear gradient solvent system of 0.1% aqueous formic acid (solvent A) and acetonitrile 0.1% formic acid (solvent B) was used as follows; 90% solvent A until 4 min, 90-75% solvent A over 25 min, 75-10% solvent A over 35 min, 90% solvent A isocratic for 15 min. The flow rate was 0.5mL/min. Compounds were monitored at a wavelength of 280 nm. Individual phenolic compounds were identified by the retention time of extract chromatographic peaks being compared with those of authentic standards using the same HPLC operating conditions. Standard compounds used to identify the phenolics present in the GE were ferulic acid, quercetin, kaempferol, ellagic acid, chlorogenic acid, vanillic acid, gallic acid, caffeic acid, p-coumaric acid, catechin, naringenin, L-ascorbic acid, myricetin and cyanidin 3-glucoside.

2.6. Antibacterial activity of GE.

Four test cultures namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Bacillus cereus* were grown overnight separately in nutrient broth (HiMedia, India). The medium was centrifuged and the supernatant was discarded and the pellet obtained was washed with saline. The pellet was then suspended in PBS and the antibacterial activity was determined using 96-well ELISA plates. Different concentrations of GE were used and proper positive and negative control was maintained. Within each sterile 96-well plate, the first row contained 50 µL media (NB) and PBS (150 µL) only (serving as a sterility check). The second row had media (50 µL), PBS (150 µL) and culture (50 µL, 105cfu/ml). In the third row each well contained media (50 µL), PBS (145 µL) and culture (50 µL) and ethanol (5 µL) (serving as the ethanol vehicle control). The following rows contained (50 µL), PBS (145 µL) and culture (50 µL) and different concentrations of GE (5 µL). The plates were incubated overnight at 37°C and the turbidity was measured using ELISA plate reader. The experiment was replicated three times in separate plates and the same procedure was followed for all the extracts.

2.7. Shelf life extension of minced chicken meat containing GE.

2.7.1. Efficacy of GE in minimising oxidative rancidity in irradiated meat. The minced chicken meat was divided into 4 portions. To the first and second part, 70% ethanol extract of AD and FD-GE (0.1%) was added respectively. The minced meat was mixed thoroughly, packed in polyethylene pouches and irradiated at 2.5 kGy (Gamma Cell 5000, India with 60Co source). The third lot was also irradiated (2.5 kGy) but did not contain GE. The fourth portion served as the non-irradiated control sample. Irradiation of chicken samples was carried out in the chilled state. After irradiation, the samples were stored at the chilled temperature and at regular intervals lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) assay according to Alasnier et al. [18].

2.7.2. Efficacy of GE as a natural antibacterial compound in chilled minced chicken meat. The fresh boneless chicken was purchased from the local market and minced in a meat mincer

(Orbit Premium, India). The minced chicken meat was divided into three batches. To the first and second batch, 70% ethanol extract of AD and FD-GE (0.1%) was added respectively. After thorough mixing, the meat was packed in polythene pouches (700 gauge; WVTR-0.4 g/m2/day; OTR-1800 ml/m2/day) and stored at 0-3°C. The third batch which did not contain GE served as the control. The total viable count (TVC) of control and treated samples were measured immediately and at regular intervals. To determine TVC, sample (25 g) were aseptically homogenized for 1 min with sterile saline (225 ml) in a Stomacher (Seward Medical, UK). Appropriate serial dilutions of the homogenate were carried out and plated (by spread plate technique) on Plate count agar (PCA) and incubated at 28°C for 48 hours. Counts were expressed as the logarithm of the colony forming units per gram (log cfu/g) sample.

2.8. Statistical analysis.

All experiments were carried out in triplicate and the average values with standard errors were reported. Analysis of variance was conducted and differences between variables were tested for significance by Tukey's one-way ANOVA using Origin Pro 6.1 (Origin Lab Corp., USA). A statistical difference at $p < 0.05$ was considered to be significant.

3. RESULTS SECTION

3.1. Major classes of bioactive compounds in GE.

The bioactive compounds in berries consist mainly of phenolic compounds and ascorbic acid. These compounds, either individually or combined, are responsible for various health benefits of berries. Phenolics are a heterogeneous group of secondary metabolites produced by plants and can range from simple to large complex molecules [19]. The phenolic composition of the extract is affected by the extraction method and the solvent used. Different solvents, either separately or in combination have been used as compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent [20]. The effect of solvent and extraction method on the phenolic content of GE is depicted in Table 1. In GE the total phenolic content was in the range of 65-150 mg/g. As compared to FD-GE, the phenolic content in AD-GE was higher. The highest phenolic content was in AD-gooseberry extracted with 70% ethanol while the lowest was in FD-gooseberry extracted with absolute ethanol (Table 1). Ethanol has been known as a good solvent for polyphenol extraction [20]. The combined use of water and organic solvent may facilitate the extraction of chemicals that are soluble in water and/or organic solvent. Luqman and Kumar [21] reported that the total phenolics in aqueous and ethanolic extracts were 336 and 318 µg GAE/mg respectively. The amount of total phenolics reported in Indian gooseberry by other researchers were in the range of 130-240 mg/g [8, 22]. Flavonoids are the most abundant polyphenol present in our diet and account for more than half of the eight thousand phenolic compounds identified. Effect of drying and solvent used for the preparation of extract on the total flavonoid content was the same as that of total phenolics (Table 1). Total flavonoid content in AD-gooseberry extracted with 70% ethanol was almost double as compared to in FD-gooseberry extracted with absolute ethanol. Quercetin is the major flavanol reported in gooseberry. Tannins are another major class of polyphenols in our diet. Unlike phenolic and flavonoid content, the tannin content was higher in aqueous extracts as compared to ethanolic extracts of gooseberry. Two hydrolyzable tannins Emblicanin A and B, and Phyllemblin have been identified in GE [23]. With thermal treatment, an increase in ellagic acid released from ellagitannins has been reported in raspberry jams [24].

Ascorbic acid is an essential micronutrient for man and acts as an antioxidant by directly scavenging reactive oxygen species and also by regeneration of other antioxidants such as tocopherol. Ascorbic acid content in GE was in the range of 169-513 mg/100g and the content varied with the solvent used for extraction (Table 1). Amongst the three solvents used 70% ethanol was most efficient in extracting ascorbic acid while aqueous extract had significantly lower ascorbic acid content. Ramful et al. [25] classified fruits into low (<30 mg/100g), medium (30–50 mg/100g) and high (>50 mg/100g) depending on their ascorbic acid content. According to this classification, GE qualified as fruit with high ascorbic acid content. Method of drying of gooseberry had less impact on the ascorbic acid content. The presence of tannins in GE prevents the oxidation of ascorbic acid even in dried fruit [26]. Methakhup et al. [9] have reported that the ascorbic acid content in gooseberry to be around 1.04 g/100g and 64-98% of it was retained under different drying conditions. Ascorbic acid content in Cape gooseberry was found to be in the range of 33.4-39.4 mg/100g FW [27].

Table 1. Effect of solvent and drying method on the major class of bioactive compounds of Indian gooseberry.

Solvent	Total phenolics* (mg/g)		Total flavonoids (mg/g)		Total tannins (mg/g)		Ascorbic acid content (mg/100 g)	
	AD	FD	AD	FD	AD	FD	AD	FD
D/W	110.01 ± 0.98^{aA}	113.72 ± 0.77^{aB}	6.14 ± 0.33^{aA}	8.86 ± 0.10^{aB}	2.63 ± 0.17^{aA}	3.28 ± 0.09^{aB}	168.0 ± 0.98^{aA}	169.5 ± 0.32^{aA}
70% ethanol	149.20 ± 0.85^{cB}	102.30 ± 0.68^{bA}	9.64 ± 0.16^{cB}	6.28 ± 0.15^{bA}	2.26 ± 0.07^{bB}	2.0 ± 0.11^{bA}	504.0 ± 0.57^{cA}	512.5 ± 0.55^{cB}
100% ethanol	123.00 ± 1.01^{bB}	65.58 ± 0.99^{aA}	7.48 ± 0.19^{bB}	4.77 ± 0.09^{aA}	1.39 ± 0.10^{aA}	1.55 ± 0.25^{aA}	459.7 ± 0.69^{bA}	473.8 ± 0.47^{bB}

* Values are given as mean ± SD (n = 3)

[a-c] Different superscript letters in each column (different solvents used for extraction) are statistically different ($p < 0.05$)

[A-B] Different superscript letters between air dried (AD) and freeze dried (FD) are statistically different ($p < 0.05$)

3.2. HPLC of GE.

Plant extracts generally occur as a combination of different types of phytochemicals and their identification is important to determine their bioactive properties. The bioactive compounds in GE were identified and a peak assigned based on the comparison of their retention times with those of known standards (Fig. 1). Using known standards, it was confirmed that the retention time of ascorbic acid was 6.3 min (peak 1), gallic acid was 8.84 min (peak 2), catechin was 19.90 (peak 3), caffeic acid was 26.53 min (peak 4), vanillic acid was 27.81 (peak 5), chlorogenic acid was 29.01 (peak 6), ellagic acid was 33.65 (peak 7), ferulic acid was 35.02 (peak 8) and kaempferol was 39.15 (peak 9). From Fig. 1 it can be seen that the HPLC profile of FD and AF-GE was not the same both quantitatively and qualitatively. In AD-GE the number of phenolics identified was more as compared to FD-GE. Ascorbic

acid and caffeic acid was the major compounds identified in AD-GE. Vanillic acid (peak 5), chlorogenic acid (peak 6) and ellagic acid (peak 7) were not detected in FD-GE. The lower antioxidant activity exhibited by FD-GE in the various in vitro antioxidant assays (Fig. 2) may be attributed to the absence of these phenolic acids. Kumar et al. [28] reported that the major phenolics identified in 70% ethanol extract of Indian gooseberry were gallic acid and tannic acid. Gallic acid was also found to be a major compound in the ethyl acetate extract of Indian gooseberry [29]. Lugasi et al. [30] reported quercetin (9.1 mg/kg) to be present in gooseberry. Filipiak-Szok et al. [31] found that the predominant phenolic acid in Indian gooseberry was gallic acid, caffeic acid, coumaric acid, and rutin.

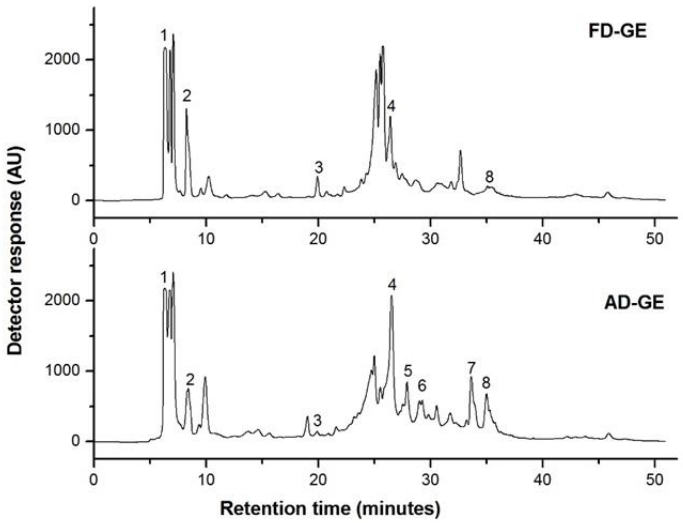

Figure 1. HPLC profile of air-dried gooseberry extract (AD-GE) and freeze-dried gooseberry extract (FD-GE).

3.3. Antioxidant activity of GE.

Antioxidant activity of crude plant extracts is not generally estimated based on a single assay as a single assay cannot mimic the complex multifunctional antioxidant mechanisms of natural extracts. Among free radical scavenging methods, DPPH assay is a simple, rapid and inexpensive test for determining antioxidant potential. AD-GE had better DPPH radical scavenging activity as compared to FD-GE (Fig. 2[A]). Amongst the solvents used in both AD and FD-GE, aqueous extract had the lowest IC50 of 10.77 and 28.04 µg/ml respectively. 70% ethanol extract of gooseberry also had low IC50 and hence antioxidant activity was comparable to aqueous extract. However, GE prepared in absolute ethanol had lowest DPPH scavenging activity as they had high IC50 of 31.25 and 44.76 µg/ml in AD and FD-GE respectively. Liu et al. [8] have reported IC50 values in the range of 11-45 µg/ml in methanolic extracts of gooseberry fruit from six regions in China. Hossain et al. [32] reported that at a concentration of 0.2 mg/mL of ethanolic extract, the DPPH scavenging activity was highest (93.7%). Apparently gallic acid was the phenolic compound that contributed most to DPPH radical scavenging activity of GE [1].

Superoxide radical by itself is not very reactive species but its conversion to OH· catalyzed by transition metals accounts for its toxicity. Scavenging of superoxide radical by GE was investigated (Fig. 2[B]). In case of FD-GE, solvent system used for extraction did not significantly have any effect on the IC50 for superoxide radical scavenging activity. However, the IC50 of aqueous and 70% ethanol extract was much lower (62.50 µg/ml) as compared to that of absolute ethanol extract (103.73 µg/ml).

Also, since the IC50 of both FD and AD samples were similar it can be concluded that the drying method did not have a significant effect on the superoxide radical scavenging activity.

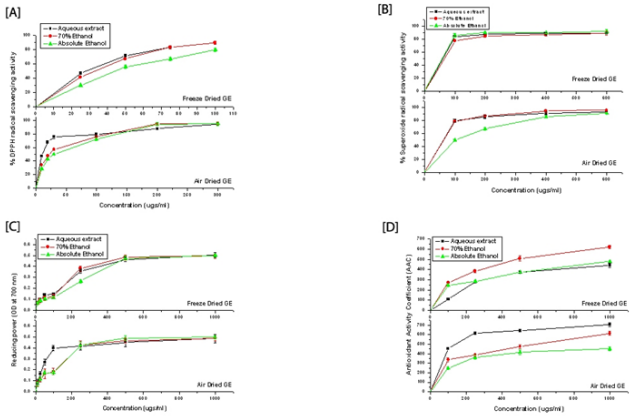

Figure 2. Antioxidant activity of GE as measured by [A] - DPPH radical scavenging activity, [B] - Superoxide radical scavenging activity, [C] - Reducing power and [D] - Beta carotene bleaching assay. Results are mean of three independent experiments.

Reducing power is associated with the antioxidant potential of plant extracts as they act as electron donors and reduce oxidized intermediates of lipid oxidation. In this assay, an increase in absorbance indicates a higher reduction of Fe3+ to Fe2+. The reducing power of GE is shown in Fig. 2[C] and it can be seen that concentration had a significant effect. Even at a low concentration of 100 µg/ml, the absorbance was in the range of 0.1 - 0.2. The solvent used for extraction or the drying method did not have any significant effect on the reducing power of GE (Fig. 2[C]). Both the AD and FD-GE extracted with all the three solvents had similar reducing power at all the concentrations used.

Beta-carotene bleaching assay is one of the best methods for assessing the antioxidant activity of natural extracts. In the AD-GE, water extract showed maximum activity while the absolute ethanol extract had only about half its activity. However, in the FD-GE 70% ethanol extract showed maximum activity (Fig. 2[D]).

3.4. Antibacterial activity of GE.

Investigation of antibacterial activity of crude extracts of plant origin against pathogens and spoilage micro-organisms assists in the development of functional food ingredients and pharmaceutical products. From the data presented in Fig. 3 it can be observed that different bacterial species exhibited different sensitivities towards GE. The antibacterial activity of GE was more against Gram-positive as compared to Gram-negative organisms studied. In case of Gram-positive organisms, it was seen that at all the concentrations investigated AD-GE was more effective in inhibiting their growth as compared to FD-GE. Amongst the solvents used it was observed that aqueous GE had the least antibacterial activity. Bobinaite et al. [33] also reported that antimicrobial properties of the raspberry extracts was affected by the solvent used for their isolation. Aneja et al. [34] found that gooseberry extract had antibacterial activity against *S. aureus* and *S. mutans* and postulated that the possible reason for the antibacterial activity might be due to the tannins present in it.

Several components of *P. emblica* fruits may act as antimicrobial agents. Nohynek et al. [35] studied the antimicrobial activity of 12 Nordic berries against selected microbes and reported that the most sensitive bacteria to berry extracts were H. pylori and B. cereus, whereas *C. jejuni* was inhibited only by phenolic extracts of cloudberry, raspberry, and strawberry, which all were rich in ellagitannins. In case of Gram-negative bacteria, it was seen that FD-GE had higher percentage growth inhibition. Water extract of FD-GE was more effective against *E. coli* in comparison to ethanolic extracts (Fig. 3). Antimicrobial activity of different berries is attributed to multiple mechanisms and synergies as they are rich in different bioactive compounds such as anthocyanins, phenolic acids and their mixtures of different chemical forms. Generally, crude plant extracts are active against a range of microbes however Gram-positive bacteria are more susceptible as compared to Gram-negative bacteria. Mechanisms responsible for antibacterial activity include both membrane and intracellular interactions of the bioactive compounds.

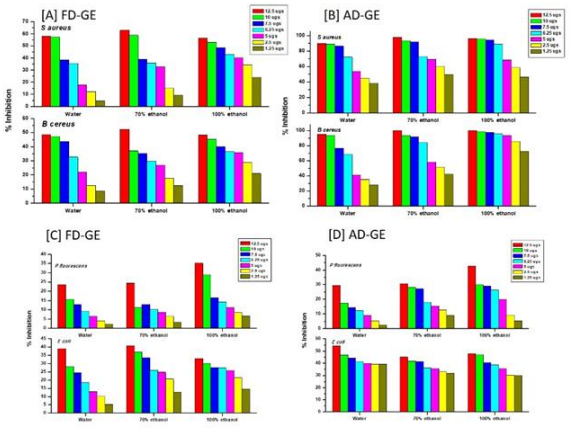

Figure 3. Antibacterial activity of [A] - FD-GE & [B]- AD-GE against *S. aureus* and *B. cereus;* [C] FD-GE & [D]- AD-GE against *P. fluorescens* and *E. coli*. Results are mean of three independent experiments.

3.5. Potential of GE as a natural additive in shelf life extension of chicken meat during chilled storage.

Fresh meat products are generally marketed at refrigerated temperatures. During refrigerated storage spoilage of meat may occur due to microbial growth and oxidative rancidity. Spoilage of fresh poultry meat is an economic burden to the poultry processing industry.

3.5.1. Antioxidant potential of GE in minced chicken meat. The most common form of chemical deterioration is the oxidation of meat lipids. It leads to the formation of several compounds which have negative effects on the sensory (color, texture, and flavor) and nutritional quality. Lipid oxidation can be reduced or inhibited by the use of antioxidants. Synthetic antioxidants have been widely used in meat and poultry products. But the consumer demand for natural antioxidants, especially of plant origin has increased in the recent years. Radiation processing is known to accelerate lipid peroxidation [36]. To establish the antioxidant potential of GE, the minced chicken was irradiated in presence or absence of GE and stored in chilled conditions. The oxidative rancidity was measured at regular intervals in terms of TBARS. TBARS indices were significantly ($p < 0.05$) affected by storage time in all the samples (Fig. 4). The highest rate of increase in TBARS values was observed in irradiated meat not containing GE. Amongst AD and FD-GE from Fig. 4, it can be seen that AD-GE was significantly more efficient in minimizing oxidation caused due to irradiation. The results of the present study show that adding phenolic-rich GE protects chicken meat against lipid oxidation. Even after 12 days of chilled storage, the TBARS value in irradiated meat containing AD-GE was around 1.5 mg MDA/kg meat which is lesser than the limiting threshold for the acceptability of oxidized meat [37]. Our results on the antioxidant activity of GE are in accordance with Erkaya et al. [38] who found that addition of Cape gooseberry to ice-cream improved its chemical and sensory properties.

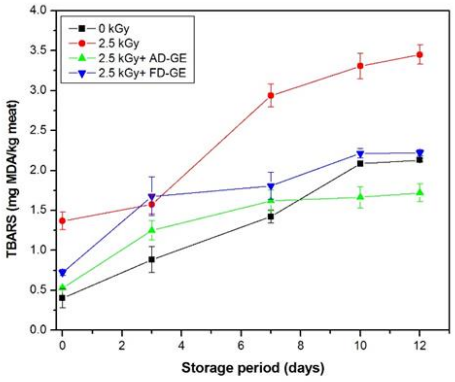

Figure 4. Lipid peroxidation as measured by TBARS in irradiated minced chicken meat during chilled storage. Results are mean of three independent experiments.

3.5.2. Antibacterial activity of GE in minced chicken meat.

Minced chicken is used in the preparation of several ready to cook products such as meatballs, sausages, burger, patties, kebabs etc. To reduce the microbial risk generally, synthetic antimicrobials are used by the meat industry. The antibacterial activity of GE and its effect in shelf life extension of minced chicken meat stored in the chilled state was investigated (Table 2). Control chicken meat (not containing GE) spoiled within 3 days as their total bacterial counts (TBC) had reached almost 7 log cfu/g, the upper acceptability limit for fresh meat [39]. On the other hand, chicken incorporated with GE had TBC of only about 6 log cfu/g after 12 days of chilled storage indicating its antibacterial activity. Addition of GE to minced chicken could delay its spoilage by 9 days as compared to control which had a shelf life of only 3 days during chilled storage. Nanasombat et al. [40] reported similar results in raw ground pork where they found that GE was effective in decreasing the TBC and Pseudomonas spp. Method of drying did not have any effect on the antibacterial activity of GE as both AD and FD-GE gave a similar effect. Using berries as a natural preservative will enable meat industry to employ a new preservation technique that will minimize the use of salt, smoke and chemical additives [41].

Table 2. Shelf life of minced chicken meat with/without GE during chilled storage.

Storage period (days)	Control chicken	Chicken with AD-GE	Chicken with FD-GE
0	5.72 ± 0.11^{a}	4.80 ± 0.11^{b}	4.89 ± 0.14^{b}
3	6.99 ± 0.15^{a}	4.85 ± 0.13^{b}	4.97 ± 0.19^{b}
7	NA	4.95 ± 0.10^{a}	5.10 ± 0.05^{a}
10	NA	5.35 ± 0.18^{a}	5.51 ± 0.11^{a}
12	NA	5.89 ± 0.19^{a}	6.10 ± 0.12^{a}

NA-Not analysed as the sample was visibly spoiled and had a bad odour. Values (mean ± SD) are average of samples analyzed

individually in triplicate; Different superscript letters within the same row indicate significant ($p< 0.05$) differences of means.

4. CONCLUSIONS

This study demonstrates the potential of GE in minimizing microbial growth, oxidative rancidity and thus extending the shelf-life of raw minced chicken meat during chilled storage for 12 days. Air drying of gooseberry and extraction in 70% ethanol gave a maximum yield of bioactive compounds. Air drying of gooseberry is much cheaper than freeze drying and hence can be used in a more economical way by the food industry. Development of methods that are acceptable to the consumer for prolonging the shelf-life and overall safety/quality of fresh chicken is a major challenge. The excellent antioxidant and antibacterial activity of GE can be exploited for the development of novel and functional meat products. Use of this natural additive will provide the meat industry greater opportunities for expansion of sale of healthy and safe meat products.

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