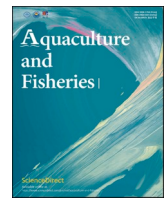




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## Effects of green tea- and amla extracts on quality and melanosis of Indian white prawn (*Fenneropenaeus indicus*, Milne Edwards, 1837) during chilled storage

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## ABSTRACT

Effect of ethanol extracts of green tea (*Camellia sinensis* L.) and amla (*Phyllanthus emblica* Linn) were investigated on quality and melanosis of chilled stored Indian white prawn (*Fenneropenaeus indicus*) during 28 days. Extracts were subjected to antioxidant assays viz. 1,1-diphenyl-2-picryl hydrazyl radical reducing power methods (DPPH), total antioxidant capacity (TAC), total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) to evaluate antioxidant potentiality and fourier-transform infrared spectroscopy (FT-IR) to identify organic constituents. Polyphenol oxidase (PPO) inhibition was assessed to check the efficacy of the extracts as anti-melanogenic agents. Biochemical (total volatile nitrogen, free fatty acid and peroxide values), bacteriological (aerobic counts), melanosis inhibition and sensory quality of chilled stored shrimp were addressed to investigate the efficacy of extracts as preservative and anti-melanogenic remedy. Free reducing power of green tea - and amla extracts were in a range of 28.72–65.67% and 17.38–66.95%, respectively. Phenolic content level was almost same for green tea and amla extract ( $2.46 \pm 0.002$  and  $2.51 \pm 0.036$  mg GAE/gram). Total antioxidant capacity of green tea ( $210.33 \pm 4.63$  mg EqAsc/g) was slightly higher than that of amla extracts ( $145.56 \pm 1.98$  mg EqAsc/g). FRAP value revealed that green tea ( $477.49 \pm 3.25$  mgE Fe (II)/g) had more ferric reducing power than amla ( $324.39 \pm 5.85$  mgE Fe (II)/g). FT-IR analysis revealed the presence of essential organic bioactive compounds, which play an important role in reducing lipid oxidation and quality loss, and both extracts possess an encouraging PPO inhibition ability. Treatment by green tea - and amla extracts on chilled stored shrimp showed promising effects on biochemical and microbiological parameters followed by melanosis inhibition and enhanced sensory attributes. Treated Indian white prawn with green tea - and amla extract revealed significantly ( $P < 0.05$ ) lower value of biochemical indices and microbial load during chilled storage compared to untreated sample.

### 1. Introduction

One of the most preferred seafood item is shrimp, with an estimated production of 4 million tonnes in 2018-19 (FAO, 2019), and accounts for 41.1% in quantity and 68.46% of total income in Indian seafood export trade (MPEDA, 2017-18). Indian white prawn (*Fenneropenaeus indicus*, Milne Edwards, 1837) is important in coastal fisheries, as well as it has an important position in the export seafood market in the tropics and subtropics due to its high price (MPEDA 2015). Furthermore, Indian white prawn is considered as an important species in the rice field shrimp farming (pokkali) of the Kerala coast of southwest India (Ranjith, Karunakaran, & Sekhar, 2018). It contains a high amount of

polyunsaturated fatty acids e.g. n-3 (204 mg/100g shrimp meat) and n-6 (106 mg/100g shrimp meat), essential amino acids, vitamin B<sub>12</sub>, selenium, zinc and other vital micro -and macro minerals, and a high protein content (19g/100 g shrimp meat) (Dayal et al., 2013). However, compared to poultry products, shrimp has very short storage life due to its biochemical composition (lipid and amino acids), muscle type and microbiological degradation due to higher muscle-pH than meat, and the microbiota. Rapid autolysis and lipid oxidation via enzymatic and non-enzymatic pathways and black spot (melanosis) formation are other major concerns for their limited shelf life. Melanosis is the oxidation of phenol to quinone in the presence of polyphenoloxidase (PPO) which undergoes polymerisation to form black spots on head, exoskeleton and

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uropod, as it causes loss in seafood market (Martinez-Alvarez, Montero, & del Carmen Gómez-Guillén, 2005) due to unappealing appearance from the consumers point of view.

To combat these problems, the industry has been dependent on synthetic chemicals, but they can cause hazard health effects. The growing health concern among consumers, has forced the processing industry to switch onto natural remedies to prevent the quality loss of the seafood product, instead of using chemical additives, which can cause many chronic health problems and can be carcinogenic above certain levels (McEvily, Iyengar, & Otwell, 1991). Recently, Xu et al. (2017) conducted an experiment by using plant extract as a potential source of antioxidant in food. The authors noticed that extracts from different medicinal plants could be an emerging source to ameliorate the damage caused by oxidative stress by inhibiting the initiation or propagation of oxidative chain reaction; acts as free radical scavengers and they comprehended on green tea extraction technologies for natural antioxidants and their effects at cellular based level. Phenolic compound e.g. catechin, ferulic acid, lead seed (*Leucaena leucocephala*), grape seed (*Vitis vinifera*), rosemary extract (*Rosmarinus officinalis*), pomegranate peel (*Punica granatum*) extract are potent anti-melanogenic agents which can lower the PPO formation and enhance the storage time of shrimp (Nirmal & Benjakul, 2009a, 2009b, 2011a, 2011b; Gokoglu & Yerlikaya, 2008).

According to Vinson (2000) and Cheng (2004), green tea (*CamelliasinisL.*) is one of the healthiest beverages in the world, as fresh green tea leaves contains 36% polyphenols, of which catechins is the major component (Tadesse et al., 2015). In addition, epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC) are well known catechins constituents present in green tea, while other catechins viz. gallic catechin (GC), gallic catechingallate (GCG) and catechingallate (CG) are present in lesser amount and other components in green tea include; different sterols, vitamins, triterpenoids and other aroma chemicals (Vishnoi, Bodla, & Kant, 2018). The most significant property of tea catechins are their antioxidant potential that can sequester metal ions and scavenge free radicals. Some studies have revealed that green tea prevents Parkinson's disease as well as to combat against colon cancer (Koo & Cho, 2004) and improve kidney functions (Mowafy, Salem, Al-Gayyar, El-Mesery, & El-Azab, 2011).

Amla (*Phyllanthusemblica* Linn) is a well-known fruit due to its ethno medical value (Gaire & Subedi, 2014), and contains nutrients and phenolic constituents such as tannins, phyllembilic acid, rutin and curcuminoids, vitamin C and several minerals (Fujii et al., 2013). Several studies reported that amla is a potent candidate against oxidative stresses (Kim et al., 2005; Reddy, Padmavathi, Paramahansa, & Varadacharyulu, 2010; Sharma, Sharma, & Kumar, 2009; Yokozawa et al., 2007), and enables antioxidant defence mechanism, and increases the levels of antioxidant enzymes like catalase, superoxide dismutase, GSH reductase, GSH peroxidase, and GSH S-transferase (Shukla, Vashistha, & Singh, 2009).

To our knowledge, the use of amla to prevent melanosis as well as other quality loss of Indian white prawn is not investigated, in addition to the effect of ethanol extract of green tea on overall quality of chilled stored Indian white prawn. Hence, the current study address to investigate the efficacy of green tea and amla extracts on improvement of chilled stored shrimp quality with respect to biochemical, bacteriological, melanosis inhibition and sensory quality.

## 2. Materials and methods

### 2.1. Chemicals and raw materials

2,2 diphenyl 1 picryl hydroxyl (DPPH), gallic acid, ammonium molybdate, 2,4,6, tripyridyl 1,3,5 Follin ciocalteu (FC) reagent, TPTZ (2, 4, 6-tri (2-pyridyl)-s-triazine), L-DOPA, ascorbic acid, nutrient agar were obtained from HiMedia (Associated Scientific Company, Kochi, Kerala). Other analytical reagents used were procured by Merck. Dry green tea

leaves, fresh amla fruit were purchased from the local market of Kochi, and Indian white prawn packed with ice in a ratio of 2:1 was brought from the fish-landing centre at Kalamukku, Kerala.

### 2.2. Extraction of green tea and amla

Green tea leaves was extracted as described by Nirmal & Benjakul (2011a, 2011b), and properly grinded tea leaves were sieved through 80 mm mesh. Twenty five gram of the uniformly grinded powder was mixed with 1L 80% ethanol (tea powder: ethanol = 1:20, w/v) and kept in magnetic stirrer followed by water bath for 2 h (Rotex Plus, W.vengola, Kerala, India) at 40 °C. After filtering, the filtrate was subjected to rotary evaporator (IKA HB10) for 60 min at 40 °C to get concentrated filtrate in two successive batches by refluxing in ethanol for re-extraction followed by drying in a hot air oven at 40 °C for 12 h, and stored in the dark at 4 °C in an airtight glass container.

Extraction of amla was carried out as described by Ahmad, Mehmood, and Mohammad (1998) with a slight modification. Briefly, amla fruit was made seedless and chopped and kept in drier at 40 °C until properly dried and to be crushed, and 10g crushed amla powder was then mixed with 50 mL of 95% ethanol (aml powder: ethanol = 1:5, w/v) and stirred continuously to obtain the maximum solvent dissolved constituents. Filtrate was thereafter concentrated by using rotary evaporator for 20 min at 40 °C under reduced pressure followed by two times re-extraction. Concentrated amla extract, was further subjected to a hot air oven for proper drying at 45 °C for 12 h, and thereafter stored at 4 °C in the dark in an airtight glass container, until further use.

### 2.3. Phytochemical screening of the extracts

Extracts were subjected to Phytochemical screening to determine the presence of tannins and saponins. Ferric chloride test and Frothing Test were performed to check out the presence of tannin and saponin respectively by the method described by Auwal et al. (2014). For tannin test, 2 mL of the ethanolic extracts were added to a few drops of 10% Ferric chloride solution (light yellow). The appearance of blackish blue colour confirmed the presence of Gallic tannins and a green-blackish colour indicated presence of catechol tannins. On the other hand, for Frothing Test, 3 mL of the ethanolic extracts were mixed with 10 mL of distilled water in a clean dry test tube and closed it with a stopper and shaken vigorously for about 5 min, it was kept for 30 min at room temperature and checked for a presence of honey - comb froth, which was the indication of the presence of saponins.

To check the presence of flavonoid presence, both the extracts were subjected to Ferric chloride test and Sodium hydroxide test. For the former one, about 0.5 of each portion was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group (Trease & Evans, 2002, pp. 95–96). For the alter one, few quantity of the each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease & Evans, 2002, pp. 95–96).

### 2.4. Antioxidant activity evaluation

#### 2.4.1. DPPH scavenging assay

Free radical scavenging ability of both the extracts was assessed by the DPPH assay as described previously (Ohnishi et al., 1994). Eight concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 80 µg/ml, 100 µg/ml, 120 µg/ml, 150 µg/ml) of pure concentrated extracts were mixed with 3 mL DPPH (0.1 mM/mL in ethanol) solution followed by 30 min incubation in dark condition. Absorbance of the mixture was recorded at 517 nm using UV-Spectrophotometer (UV-1800

SHIMADZU). Ascorbic acid was used as standard and radical scavenging percentage was determined as described by [Ohnishi et al. \(1994\)](#) using the following formula,

$$\% \text{ of DPPH scavenging} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

#### 2.4.2. Total phenolic content (TPC) determination

Estimation of total phenolic content present in the extracts were estimated spectrophotometrically by using Follin ciocalteu (FC) reagent following the standard method described by [McDonald, Prenzler, Antolovich, and Robards \(2001\)](#) with slight modifications. In the present study, gallic acid was used to obtain standard calibration curve. One mL of aliquots were added 5 mL FC reagent and sodium carbonate (4 mL, 0.7M) followed by 30 min absorbance period. Absorbance values were recorded at 765 nm using spectrophotometer and the standard curve was plotted according to [McDonald et al. \(2001\)](#)

Phenolic content (T) of both the extracts was expressed in mg GAE/g. The following formula was used to calculate the TPC,

$$T = CV/M$$

Where C = Gallic acid concentration (mg/ml) from plotted standard curve; V = volume of extract taken (ml); M = weight of sample (g).

#### 2.4.3. Total antioxidant activity (TAC) evaluation

Determination of total antioxidant capacity was performed by the phosphomolybdenum method as reported previously ([Prieto, Pineda, & Aguilar, 1999](#)). 0.3 mL of aliquot from each extract (1 mg/mL) was added 2.7 mL of phosphomolybdenum reagent, which was prepared with 28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid followed by an incubation of 90 min at 95 °C in a water bath. Thereafter, it was cooled to room temperature, and absorbance recorded at optical density (OD), OD<sub>695</sub>. A blank sample (without adding any test sample) was analysed simultaneously along with green tea and amla test samples. TAC results were expressed as equivalent of standard ascorbic acid (mg Asc/g of dry sample) using the ascorbic acid standard calibration curve.

#### 2.4.4. Evaluation of ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power of green tea and amla extracts were analysed spectrophotometrically by the procedure described by [Benzie and Strain \(1996\)](#). The FRAP reagent was prepared by mixing acetate buffer (300 mmol/L, pH 3.6), 10 mmol/L TPTZ solution in 40 mmol/L HCl and 20 mmol/L FeCl<sub>3</sub> solution in proportions of 10:1:1 (v/v), respectively. Freshly prepared FRAP reagent was warmed to 37 °C in a water bath before use. The samples were added to the FRAP reagent. The absorbance of the reaction mixture was than recorded at OD<sub>593</sub> after 4 min FeSO<sub>4</sub> was used to prepare the standard curve. The results were expressed as mgEq Fe (II)/g dry weight of extracts.

#### 2.4.5. FT-IR analysis of green tea and amla ethanol extract

Green tea and amla ethanol extracts were scanned in the range of 4000-400/cm with a resolution of 4/cm. The FT-IR spectra of these two samples were recorded in FT-IR spectrometer (Thermo Nicolet, Avatar 370) using DTGS detector and the KBr beam splitter with ZnSe crystal at 45° angle (Thermo Nicolet). Two mg of each sample was used with respective extraction solvents, and terminated after 20 s. An average of three scans was used to minimise the error.

#### 2.4.6. Assessment of green tea and amla extract efficacy on polyphenols oxidase (PPO) activity

**2.4.6.1. Crude PPO extract from shell of shrimp.** PPO isolation was carried out as described by [Simpson, Marshall, and Otwell \(1987\)](#) with slight modification. Briefly, shell of 30 shrimps was separated, cleaned with potable water and grinded in cooled condition, and kept at -20 °C

for 15 days. 50 g of grinded shell was mixed with 150 mL extracting buffer (0.05 M Sodium phosphate buffer having 1 M NaCl and 0.2% Brij-35, pH 7.2). The mixture was stirred for 30 min at 4 °C by using a magnetic stirrer followed by centrifugation at 8.000 rpm at 4 °C for 30 min. Supernatant was collected, added ammonium sulphate until it reached 40% saturation. Thereafter, it was stored for 30 min at 4 °C, and centrifuged at 12.500 rpm at 4 °C for 30 min. Pellet was collected and added with minimum quantity of 0.05 M sodium phosphate to get dissolved and thereafter centrifuged once more at 3.000 rpm at 4 °C for 30 min. Supernatant was collected and used as "Crude PPO extract".

#### 2.4.6.2. Inhibitory activity of green tea extract and amla extract on PPO.

Inhibitory activity was carried out as described by [Nirmal & Benjakul \(2009a, 2009b\)](#). Hundred µL of five different concentrations of GTE and AE (0.2%, 0.4%, 1%, 2% and 4% w/v) was mixed with 100 µL of prepared crude PPO extract to get the final concentrations of 0.1%, 0.2%, 0.5%, 1% and 2% w/v respectively and incubated for 30 min at room temperature. 400 µL of phosphate buffer was then added which was followed by 600 µL of pre incubation (45 °C) with 15 mM L-DOPA. Reaction mixture was left for 3 min at 45 °C and absorbance measured at 475 nm. Control used was without extract. Relative activity, expressed as percentage was calculated using the following formula:

$$\text{Relative activity } (\%) = \frac{\text{PPO activity in the presence of extracts} \times 100}{\text{PPO activity of control}}$$

#### 2.5. Shrimp sample preparation

Whole Indian white prawns were divided into three slots; green tea treated -, amla treated -, and control slot (without any treatments). For the green tea - and amla treated slots, 50 g/L extracts were used, and soaked for 15 min. Treated shrimps were drained for 3 min at 4 °C and thereafter all the six slots were packed separately in 12µ polyester laminated with polyethylene bags of 20 × 15 cm dimension and 420 mm thickness and stored at 4 °C throughout the experiment.

#### 2.6. Proximate composition analysis of Indian white prawn

All proximate analysis was performed by standard methods of [AOAC \(2005\)](#); moisture content analysis (AOAC – 925.10), total fat content by soxhlet extraction (AOAC – 2003.05), ash content by combustion procedure (AOAC – 923.03) and protein by the micro Kjeldahl method.

#### 2.7. Chemical analyses

##### 2.7.1. Total volatile base nitrogen content

TVB-N determination was done using Conway micro-diffusion method ([Conway, 1933](#)) and value obtained in the experiment was expressed as mg TVB-N/100 g muscle.

##### 2.7.2. Free fatty acid (FFA) content

Free fatty acid analysis of the samples was carried out by the standard protocol of [AOAC \(1975\)](#) and the results expressed as percentage of oleic acid.

##### 2.7.3. Peroxide value (PV) evaluation

Peroxide value was assessed by standard [AOCS \(1989\)](#) protocol and value was expressed as milliEquivalents/kg of fat.

#### 2.8. Bacteriological analyses

##### 2.8.1. Aerobic bacterial count

Twenty-five gram of aseptically minced shrimp samples were transferred to stomacher bags, containing 225 mL of 0.85% physiological sterile saline, and mixed for 2 min in a stomacher blender until uniformity. Hundred µL of 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> dilutions

were plated on nutrient agar plates with 0.5% salt, and incubated at  $35^{\circ} \pm 2^{\circ} \text{C}$  for  $48 \pm 2 \text{h}$  for measuring aerobic plate count (mesophilic bacteria) as described by [Songsaeng, Sophanodora, Kaewsrithong, and Ohshima \(2010\)](#), and the values expressed as CFU/g muscle.

### 2.9. Melanosis assessment

Melanosis was assessed at every 4th day interval basis by the melanosis scale reported by [Montero, Lopez-Caballero, and Pérez-Mateos \(2001\)](#), and all samples and the control were given a specific code and each package contained ten numbers of shrimp. Score 0 represented absence of blackening; 2 represented blackening up to 20% of shrimp exoskeleton. Likewise, score 4 was assigned for moderate blackening or 20–40% of the shrimp body, 6 for 40–60% and 8 for severe (60–80% shrimp body) blackening and 10 was assigned for overall (80–100%) blackening. The same personnel assigned for melanosis evaluation also evaluated sensory quality evaluation.

### 2.10. Sensory evaluation

Sensory evaluation was carried out, every 4th day interval basis according to the 9-point hedonic scale ([Meilgaard, Civille, & Carr, 1999](#)). All samples and the control were given a specific code and each package contained ten shrimp. Score 9 represented the superior quality or like extremely and 1 represented the poorest quality or dislike extremely. Likewise, 7 point for moderate likeness, 5 for neutral likeness and 3 signified moderately disliking. Sensory quality was assessed by a 5 member expert panel.

### 2.11. Statistical analyses

Each test was carried out in triplicate, and performed in a completely randomised design (CRD). The T-test used Graph pad prism 6 and SPSS package, and  $P < 0.05$  was considered as statistically significant. Mean and Standard error (SE) at 5% was level of significance.

## 3. Results and discussion

### 3.1. Phytochemical screening of the extracts

Phytochemical screening of the ethanolic extracts of green tea and amla revealed the presence of various bioactive components as summarised in [Table 1](#). It indicates the presence of tannin, saponin, flavonoid in the ethanolic extract of Green tea leaves and amla fruit. The occurrence of blackish blue colour upon adding 10% Ferric chloride solution (light yellow) showed the presence of tannin in green tea and amla. Similarly honey-comb frothing of GTE and Amla extract while shaking vigorously with 10 ml distilled water for about 5 min and allowed to stand for 30 min indicated the presence of saponin. For flavonoid qualitative test, Ferric chloride test and Sodium hydroxide test both were performed and they showed positive result as green-blue colouration appeared for both extract upon adding few drops of 10% ferric chloride solution and a change in colour from yellow to colourless

**Table 1**  
Phytochemical screening of Green tea and amla ethanolic extract.

Phytochemical constituent	Type of test	Appearance	Green tea extract	Amla extract
Tannin	Ferric chloride test	Blackish blue	+	+
Saponin	Frothing test	Honey comb like froth	+	+
Flavonoid	Ferric chloride test (10%)	Green blue	+	+
	Sodium hydroxide test	Yellow to colourless	+	+

on addition of dilute hydrochloric acid was an indication for the presence of flavonoid.

### 3.2. Antioxidant capacity of green tea and amla extract

#### 3.2.1. DPPH scavenging assay

The DPPH scavenging assay is an effective antioxidant assay as it confirms the ability of the extracted components to scavenge free radicals ([Ohnishi et al., 1994](#)). This is based on hydrogen donation as donor of hydrogen molecule acts as an antioxidant agent, and compounds can be measured as free radical scavengers and DPPH free radical has the ability of transferring electron and producing violet colour solution in methanol ([Poli et al., 2003](#)). The degree of radical-scavenging potential of the samples was denoted by the degree of discolouration of the solution according to [Blois \(1958\)](#). DPPH scavenging percentage ability and IC 50 (effective concentration) of green tea and amla ethanol extracts are shown in [Tables 2 and 3](#), respectively. The different concentrations of green tea extract (10–150  $\mu\text{g/mL}$ ) showed a range of percentage inhibition between 28.7% and 65.7%, whereas amla at similar concentrations revealed percentage of radical scavenging ability between 17.4% and 66.9%. The result of the present study shows a parallel correlation of scavenging ability with the highest extract concentrations, and is in accordance with [Kumari and Khatkar \(2016\)](#). They reported IC 50 values for antioxidant activity of amla fresh fruit within a range of 46.7–359.7  $\mu\text{g/mL}$ . [Nanjo et al. \(1996\)](#) reported the highest antioxidant capacity for ethanol extract followed by aqueous extracts for green tea. [Manjulatha, Manjari, Sarita, Imam, and Setty \(2014\)](#) reported DPPH radicals of amla with a percentage inhibition range of  $75.59 \pm 2.98$  to  $72.25 \pm 4.63\%$  at the highest concentration of 200  $\mu\text{g/mL}$  and IC50 values range of 41.05 to 32.66  $\mu\text{g/mL}$ , which support the results of the present study. The present study revealed that both extracts have appreciable level of free radical scavenging ability. Hence, it shows the potentiality to be natural antioxidant to reduce oxidative damage during chilled storage.

#### 3.2.2. Total phenolic content (TPC)

Phenolic compound is the largest and ubiquitous group among all plant metabolites, which have aromatic ring possessing hydroxyl group ([Singh et al., 2007](#)). However, a previous study revealed that, natural antioxidant comprises of different types of polyphenols mainly flavonoid groups along with phenolic acid and tocopherol in plant source ([Ali et al., 2008](#)). These phenolic groups play the pivotal role in scavenging reactive oxygen constituents ([Pourmorad et al., 2006](#)).

The gallic acid standard solution of different concentration (25–200  $\mu\text{g/mL}$ ) was subjected at 765 nm. A regression co-efficient ( $R^2$ ) = 0.998 and a slope (m) = 0.003 were obtained with an intercept = 0.187. The equation of standard curve is  $y = 0.003x + 0.187$  ([Fig. 1](#)). The total phenolic content of green tea and amla both ethanol extract were found to be promising with a value of  $2.4699 \pm 0.002$  mg Eq GAE/g dry weight of green tea extract and  $2.5151 \pm 0.036$  mg Eq GAE/g dry weight of amla extract ( $p < 0.05$ ). Total phenolic content of green tea and amla are summarised in [Table 3](#). The present study revealed both green tea and amla have almost similar quantity of phenolic content ( $\sim 2.5$  mg Eq

**Table 2**

Percentage (%) of DPPH radical scavenging ability of green tea and amla ethanol extracts at different concentration.

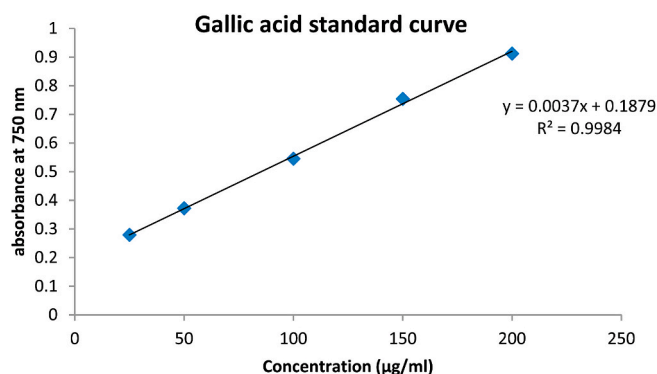
Concentration ( $\mu\text{g/mL}$ )	Green tea extract (% RS)	Amla extract (% RS)
10	28.72	17.38
20	44.83	28.91
30	48.68	37.77
50	51.48	51.29
80	54.64	55.07
100	59.54	64.67
120	62.69	65.06
150	65.67	66.95



**Table 3**  
IC50, TPC, TAC and FRAP of green tea and amla ethanol extract.

Extracts	IC 50	TPC (mg Eq Quercetin/100g)	TAC (mg EqAsc/g)	FRAP (mgE Fe (II)/g)
Green tea extract	60.33	2.46 ± .002	210.33 ± 4.63	477.49 ± 3.25
Amla extract	74.89	2.51 ± .036	145.56 ± 1.98	324.396 ± 5.85

Values are mean ± S.D, n = 10.



**Fig. 1.** Total phenolic content standard curve of Gallic acid Concentration ( $\mu\text{g/ml}$ ).

GAE/ $\text{g}^{-1}$ ).

The TPC of green tea revealed in the present study is similar to the TPC value of 2.53 mg GAE/g for green tea reported by Ramírez-Aristizabal, Ortíz, Restrepo-Aristizabal, and Salinas-Villada (2017). Haji et al. (2008) observed similar value of green tea total phenolic content ( $0.196 \pm 0.012$  mg GAE/gram) due to the high antioxidant activity of green tea. Phenolic contents from green tea extracts correlate with radical scavenging activity (Li et al., 2009), and this explains their reducing capability by hydrogen donation as well as singlet oxygen quenching. Tsai, Tsai, Yu, and Ho (2007) reported that phenolic compounds in green tea have strong antioxidant capacity, as it possesses flavan-3-ols and can prevent oxidative stress by chelating available free ferrous ions.

The TPC value of  $2.51 \pm 0.036$  of amla revealed in the present study is similar as that reported by Mukhinder (2017). Agarwal, Kumar, Gupta, and Upadhyaya (2012) reported that amla possesses a high amount of total phenolic content, and it increases with higher concentration of extraction solvent. Polyphenols content varies with solvent, degree of polarity of the solvents and compatibility of compound with the solvents (Teh et al., 2014; Zhang et al., 2007). Hossain, Barry-Ryan, Martin-Diana, and Brunton (2010) stated that fresh sample revealed lower value of TPC compared to processed fruits, due to that the outer layers contains higher phenolic content than seed or pulp (Chism & Haard, 1996). Green tea and amla extracts comprise of good amount of phenolic compounds which enriches their antioxidant activity.

### 3.2.3. Total antioxidant capacity (TAC)

Total antioxidant capacity by phosphomolybdenum method is based on the principle of green phosphate complex MO (V) formation from MO (VI) in the presence of sample analysed (Subhashini, Thangathirupathi, & Lavanya, 2011). The phosphomolybdenum method is quantitative since TAC is expressed as the numbers of equivalent of ascorbic acid. It is otherwise termed as PM (phosphomolybdenum) assay, and is widely used as a routine laboratory method to quantify the total antioxidant capacity of certain plant extract (Prieto et al., 1999). TAC values of green tea and amla ethanol extracts are revealed in Table 3. Green tea showed more total antioxidant capacity ( $210.33$  mgE Asc/g) compared to amla ( $145.56$  mgE Asc/g) ( $p < 0.05$ ). The reason for this could be the presence

of several compounds like polyphenols, ellagic acid, gallic acid and tannins in green tea. Another reason could be high antioxidant capacity, due to the presence of hydroxyl (OH) groups on the  $\beta$ -ring as in EGCG, GCG, EGC and GC in green tea, which are suggested to have more ability to scavenge free radicals (Almajano, Carbo, Jiménez, & Gordon, 2008). Haji et al. (2008) observed almost similar TAC value as that revealed in the present study, as the authors displayed that 1 g of green tea is equivalent to 50–275 mg of pure ascorbic acid (vitamin C), or 156–813 mg vitamin E, which implicate that green tea is an efficient source of antioxidant. It was previously displayed that green tea is able to inhibit lipid peroxidation by their free radical scavenging activity (Soong & Barlow, 2004).

Manjulatha et al. (2014) revealed TAC in amla in a range of  $249.81 \pm 0.63$  to  $733.02 \pm 2.1$  mg ascorbic acid equivalent/g, and is in agreement with the results of the present study. Amla comprise a higher amount of abundant antioxidant (Antony, Merina, Sheeba, & Mukkadan, 2006), and it is believed that amla can be a potent alternative source of synthetic additives to prevent lipid oxidation in food processing sector (Liu, Qiu, Ding, & Yao, 2008). Reason behind this high amount of total antioxidant may be due to the presence of various polyphenols i.e. kaempferol, ellagic acid and gallic acid (Habib-ur-Rehman et al., 2007).

### 3.2.4. Ferric reducing antioxidant power (FRAP) assay

FRAP is a widely used antioxidant assay which works based on the principal of redox-linked colorimetric reaction, where ferric ion ( $\text{Fe}^{3+}$ ) converts to ferrous ( $\text{Fe}^{2+}$ ) ion at a lower pH, and forms coloured ferrous-probe complex from a colourless ferric-probe complex (Firuzi et al., 2005). The antioxidant activity is parallel to the polyphenols content of the solvent extracts. Polyphenols are more efficient reducing agents for ferric iron (Wong, Leong, & Koh, 2006), and possess a highly positive relationship between total phenols and antioxidant activity (Oktay, Gülçin, & Küfrevioğlu, 2003). The reducing power of the ethanol extract of green tea and amla are revealed in Table 3. Amla ethanol extract showed FRAP values of  $324.39 \pm 5.85$  mgE Fe (II)/g, while green tea ethanol extract revealed  $477.49 \pm 3.25$  mgE Fe (II)/g, and the values were significantly ( $P < 0.05$ ) different. Amla sought seems to have more reducing power compare to green tea extract, and this is probably due to the high concentration of polyphenols present in amla compare to green tea (Habib-ur-Rehman et al., 2007). The present study observed a good correlation between DPPH scavenging ability, and ferrous ion reducing ability that implies the efficacy of both the extract to be a potent reducing agent to fight against oxidation. This trend is in agreement with the finding of Chen, Lin, and Hsieh (2007).

### 3.3. FT-IR analysis

In the present study, green tea - and amla ethanol extracts were subjected to FT-IR analysis. Fig. 2 a and b show the FT-IR spectral profile for green tea and amla respectively. Main peaks range from  $3271.88$   $\text{cm}^{-1}$  to  $432.30$   $\text{cm}^{-1}$  for green tea and  $3278.53$   $\text{cm}^{-1}$  to  $766.71$   $\text{cm}^{-1}$  for amla are showed with extended marking. It was revealed that  $3271.88$ ,  $1694.89$ ,  $1606.84$ ,  $1552.85$ ,  $1518.0$ ,  $1448.80$ ,  $1338.63$ ,  $1235.77$ ,  $1144.14$ ,  $1033.96$ ,  $821.78$ ,  $763.34$  and  $432.30$   $\text{cm}^{-1}$  main peaks for green tea and  $3278.53$ ,  $1719.59$ ,  $1613.68$ ,  $1544.60$ ,  $1343.10$ ,  $1213.26$ ,  $1039.34$ ,  $918.96$ ,  $871.32$ ,  $812.71$  and  $766.71$   $\text{cm}^{-1}$  main peaks for amla were clearly recorded in fingerprint region of FT-IR. In case of green tea,  $3271.88$   $\text{cm}^{-1}$ , the value could be due to vibration of  $-\text{OH}$  stretching bands (Nakashini, 1969; Silverstein et al., 1998).  $1694.89$   $\text{cm}^{-1}$  is due to  $\text{C}=\text{O}$  stretching of protein and  $1606.84$   $\text{cm}^{-1}$  is due to  $\text{CH}_2$  symmetric stretching (Toyran, Zorlu, & Severcan, 2005). Range of  $1500$ – $1600$   $\text{cm}^{-1}$  indicates the presence of flavonoids, polyphenols and catechins (Lam, Proctor, Howard, & Cho, 2005).  $1448.80$ ,  $1338.63$ ,  $1235.77$   $\text{cm}^{-1}$  is mainly due to  $-\text{CH}$  in plane bending vibration of allylic group,  $-\text{CH}$  stretching vibration of gem-dimethyl group and  $\text{C}-\text{O}-\text{C}$  stretching vibration band of ether group, respectively.  $1144.14$   $\text{cm}^{-1}$  indicates the

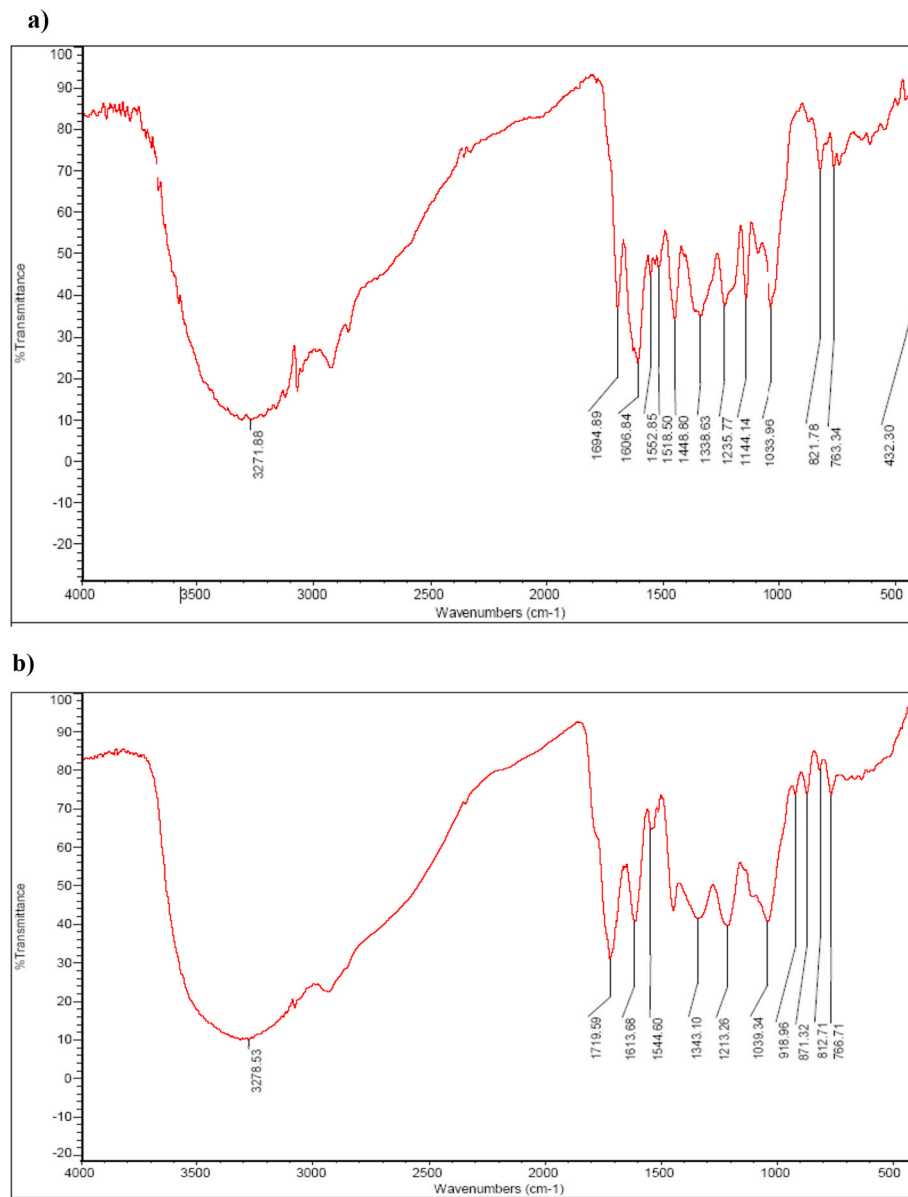


Fig. 2. a) FT-IR spectra of green tea ethanol extract sample and b) amla ethanol extract sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

presence of alcohols, esters, carboxylic acids groups in sample. Peaks of 1033.96, 821.78 and 763.34  $\text{cm}^{-1}$  could be due to the presence of  $-\text{CH}$  in plane bending vibration of trans or E-alkene, and cis or Z-alkene presence as suggested previously (Nakashini, 1969; Silverstein et al., 1998). In case of amla, 3278.53  $\text{cm}^{-1}$  is probably due to O–H stretch, H-bonded, which signifies the presence of phenols and alcohols (Orčić, Mimica-Dukić, Francisković, Petrović, & Jovin, 2011). The spectral data of 1719.99, 1613.68 and 1544.60  $\text{cm}^{-1}$  may be due to the presence of  $-\text{CH}$  symmetric/asymmetric aliphatic bond, ketones/carbonyl groups, phenyl ring and aromatic ring C–C stretching. Similarly, visual intensity estimates for the spectral band of 1343.10, 1213.26 and 1039.34  $\text{cm}^{-1}$  is mainly due to C–O (stretching) – ester, CO stretching and aromatic ring stretching. The occurrence of spectral wavelength of 918.96, 871.32, 812.71 and 766.71  $\text{cm}^{-1}$  is possible due to  $\text{HC}=\text{CH}$  aromatic amides as previously suggested by Miyazawa, Shimanouchi, and Mizushima (1956). The main chemical groups present in the extracts analysed by FTIR are summarised in Table 4.

Table 4

Chemical groups analysed in the extracts as per the main peaks obtained by FTIR.

Chemical group	Green tea extract	Amla extract
Flavonoids	+	+
Poly phenols	+	+
Catechins	+	+
Allylic	+	-
Dimethyl	+	-
Ethers	+	-
Esters	+	+
Carboxylic acids	+	-
Alkenes	+	-
Phenols	-	+
Ketones	-	+
Carbonyls	-	+
Aromatic Amides	-	+

+: Present, -: absent.

### 3.4. Polyphenols oxidase (PPO) activity

Polyphenol oxidase is an endogenous enzyme present in shellfish (McEvily et al., 1991) and the major cause of melanosis reaction causing blackening of shrimp head and cephalothorax. The effect of green tea and amla extract solution on inhibition of PPO from Indian white prawn is showed in Fig. 3. The present study revealed its efficacy in dose dependent manner ( $P < 0.05$ ). At similar concentration level, amla revealed better relative activity percentage compared to green tea ( $P < 0.05$ ). Although, at 0.2% concentration both the extract solutions showed similar results ( $P > 0.05$ ). Nevertheless, present study revealed an encouraging increase in PPO inhibition of amla extract at 2% concentration compared to the green tea extract ( $P < 0.05$ ). A previous study revealed that, interaction of enzyme with phenolic compound could prevent PPO activity (Janovitz-Klapp, Richard, Goupy, & Nicolas, 1990). Nirmal & Benjakul (2009a, 2009b) reported that catechins showed PPO inhibitory activity in a dose-dependent manner, and it was assumed that catechins might act as a competitive inhibitor for PPO due to similarity to L-DOPA structure, which is a substrate for PPO. The present study suggest that green tea and amla extract are effective to control PPO effects on chilled stored shrimp, and can be used as an alternative natural anti-melanogenic substance.

### 3.5. Proximate analysis

In the present study, the proximate composition of Indian white prawn was:  $71.60 \pm 2.54\%$  moisture,  $10.32 \pm 0.86\%$  crude protein,  $8.24 \pm 0.11\%$  crude fat,  $1.96 \pm 1.21\%$  ash. Variations in chemical composition are not unusual as Karuppasamy et al. (2013) reported variations in chemical composition of penaeid shrimp, mainly in protein and moisture.

### 3.6. Evaluation of green tea extract and amla extract effect on the quality of chilled stored Indian white prawn

#### 3.6.1. Total volatile base nitrogen (TVB-N)

TVB-N is the most reliable parameter for evaluation of freshness indices of fish and shellfish and indicates sign of spoilage with production of trimethylamine (TMA). A level of 35–40 mg TVB-N/100g of fish muscle is an acceptable limit (Lakshmanan & Fung, 2000). High TVB-N values is considered as spoiled and unfit for consumption as trimethylamineoxide (TMAO) is reduced to TMA, dimethylamine and formaldehyde by the action of endogenous enzymes or by spoilage bacteria. TMA causes off odour (Regenstein, 1982) and high levels are regarded as

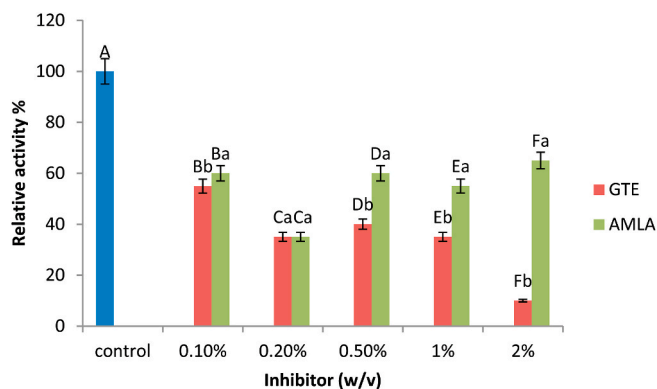


Fig. 3. Effect of green tea - and amla extract at different levels on the inhibition of polyphenoloxidase from shell of Indian white prawn. Bars represent the standard error ( $n = 3$ ). Different capital letters on the bars within the same GTE or AE together with the control indicate the significant differences ( $P < 0.05$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

unacceptable. TVB-N of all groups showed a parallel increment with storage time ( $P < 0.05$ ). At start, the initial TVB-N value was  $2.38 \pm 0.009$  mgN/100 g, but at day 28th, the control slot showed an increased TVB-N value,  $34.39 \pm 0.04$  mgN/100 g. However, on day 28th of chilled storage at  $4^\circ\text{C}$ , treatment of green tea revealed a TVB-N value of  $27.84 \pm 0.009$  mgN/100 g, and amla treated sample  $28.67 \pm 0.012$  mgN/100 g (Fig. 4), which indicates that the control is unacceptable, in contrast to treated shrimps.

In the present study, a good correlation between TVB content and the bacterial load was revealed (Table 5) and this finding correspond well with that revealed by Ozogul et al. (2010) as they displayed that untreated fish sample was declared as rejected on 13th day of chilled storage. However, chilled storage samples treated with rosemary extract was acceptable until day 17. Furthermore, the findings in the present study are in accordance with Nirmal & Benjakul (2011a, 2011b) in their study on Pacific white shrimp (*Litopenaeus vannamei*) treated with lead seed extract. Viji et al. (2015) reported a similar trend in a study with chill stored Indian mackerel and fish treated with mint (*Mentha arvensis*) leaf and citrus (*Citrus aurantium*) peel extracts.

The findings of the present study is also in agreement with previous reports observing an beneficial effect by using plant extracts to improve TVB-N values of chilled stored fish products (Gao et al., 2014; Pezeshk, Rezaei, & Hosseini, 2011).

#### 3.6.2. Free fatty acid content (FFA)

FFA is considered as an indicative tool for quality assessment of chilled stored fish and shellfish. In the current study, significant ( $P < 0.05$ ) differences ( $P < 0.05$ ) in FFA contents were observed among the treatment groups throughout the storage period (Fig. 5). In control sample, increased the FFA value from  $0.017 \pm 0.001\%$  (expressed as percentage of oleic acid) to  $0.137 \pm 0.002\%$ , whereas values of  $0.017 \pm 0.001\%$  to  $0.112 \pm 0.001\%$  were revealed for green tea treated sample, and  $0.024 \pm 0.001\%$  to  $0.115 \pm 0.001\%$  for amla treated samples (Fig. 5). This trend is in accordance with the previous finding of Gokoglu, Yerlikaya, Topuz, and Buyukbenli (2012), where FFA value of fish croquette was  $2.75 \pm 0.12\%$  in the control, while a value of  $1.83 \pm 0.01\%$  was observed in croquettes treated with tomato extract after a storage period of four months.

Free fatty acids are derived by enzymatic or non-enzymatic hydrolysis of lipids, particularly phospholipids and triglyceride, and are located primarily in the cell membrane (Serdaroğlu & Felekoğlu, 2005). Both lipase and phospholipase enzymes have significant activity by producing FFA in various fish and shellfish species stored at  $-12$  or  $-14^\circ\text{C}$  (Olley, Pirie, & Watson, 1962). In an early study, interaction during frozen storage of various fish species revealed that, accumulation of FFA increased with prolonged storage time, and at elevated frozen storage temperature (Dyer & Dingle, 1961). In the present study, it was observed a lower lipid hydrolysis rate in both treated slots irrespective of their antioxidant contents (Fig. 5). This finding is in accordance to that revealed by Sarah, Hadiseh, Gholamhossein, and Bahareh (2010) where

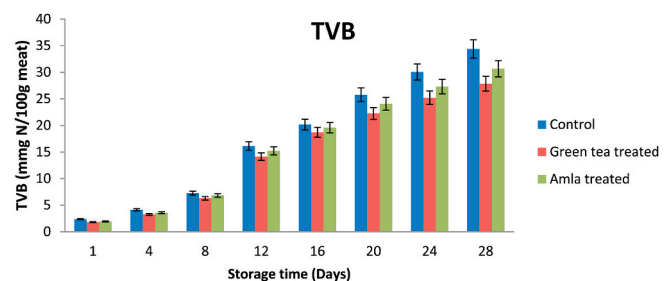


Fig. 4. Total volatile base content of Indian white prawn treated with green tea - and amla extract, and control during 28 days of storage at  $4^\circ\text{C}$ . Bars represent the standard error ( $n = 3$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 5**

Determination of aerobic bacterial counts of Indian white shrimp samples treated with green tea, amla and control during the 28 days storage study.

	1st day	7th day	14th day	21st day	28th day
Control (CFU/mL)	$3.00 \times 10^4$	$6.00 \times 10^5$	$4.02 \times 10^7$	$9.0 \times 10^7$	$2.1 \times 10^8$
Green tea (CFU/mL)	$2.03 \times 10^4$	$1.6 \times 10^5$	$2.00 \times 10^5$	$2.0 \times 10^6$	$3.4 \times 10^6$
Amla (CFU/mL)	$2.10 \times 10^4$	$3.40 \times 10^5$	$4.9 \times 10^5$	$6.7 \times 10^6$	$2.6 \times 10^7$

Values are mean  $\pm$  S.D, n = 10.

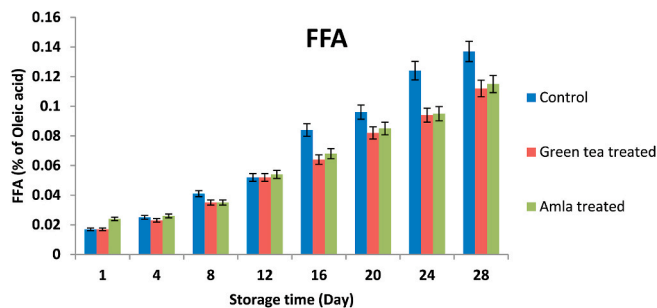


Fig. 5. Free fatty acid content of Indian white prawn treated with green tea – and amla extract, and control during 28 days of storage at 4 °C. Bars represent the standard error (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

refrigerated Persian sturgeon (*Acipenser persicus*) treated with green tea and onion juice lower the FFA content compared to the untreated group after 8 days of storage. Similar findings were reported in studies by different authors evaluating the effect of different antioxidants (Aubourg, Pérez-Alonso, & Gallardo, 2004; Serdaroglu & Felekoğlu, 2005; Chaijan, Benjakul, Visessanguan, & Faustman, 2006; Lugasi et al., 2007; Sahari, Nazemroaya, & Rezaei, 2009; Taheri, Motalebi, & Fazlara, 2012).

### 3.6.3. Peroxide value (PV)

Fish lipid is very prone to oxidation as hydroperoxides it produced due to its rich content of highly unsaturated fatty acid. It oxidises iodide to iodine or iron (II) to iron (III), can be estimated by PV evaluation (primary oxidation compounds) in terms of milliEquivalent peroxide/kg extracted fat from sample (Stine, Harland, Coulter, & Jenness, 1954). Detecting spoilage at earlier stage is difficult as this compound is odour free, but at later stages, it breaks down and forms auto-oxidative constituents, a sign of early phase of auto-oxidation, which leads to severe oxidative spoilage. Breakdown products i.e. aldehydes, ketones and alcohols are volatile in nature and causes off odour, and indicates rancid stage of product. PV plays an important role in early stage of oxidation as it increase hydroperoxide formation rate, higher than the decomposition rate, but once it reach maximum level, it starts decreasing due to lower availability of substrates and instability of peroxide molecules (De Abreu, Losada, Maroto, & Cruz, 2011). Alghazeer, Saeed, and Howell (2008) stated that green tea (250 ppm and 500 ppm) treated Atlantic mackerel (*Scomber scombrus*) fillets revealed lower peroxide and hydroperoxide rate during 8 weeks of storage at  $-10$  °C compared to control. In addition, it is worth to mention that several studies proved that plant extract could efficiently lower peroxide formation by delaying secondary reactions of the hydroperoxides (De Abreu et al., 2011; Pezeshk et al., 2011; Shi, Cui, Yin, Luo, & Zhou, 2014; Viji et al., 2015).

In the present study, PV increased from  $0.81 \pm 0.004$  milliEquivalents/kg to  $18.46 \pm 0.0004$  milliEquivalents/kg in the control group, while green tea and amla treated samples revealed a significantly ( $P < 0.05$ ) lower level of  $13.82 \pm 0.004$  and  $15.47 \pm 0.009$

milliEquivalents/kg from the initial value of  $0.66 \pm 0.008$  and  $0.68 \pm 0.0004$  milliEquivalents/kg, respectively. These PV values were significant ( $P < 0.05$ ) different between Indian white prawn treated with green tea and amla extracts and control (Fig. 6). Haghparast, Kashiri, Alipour, and Shabanpour (2011) reported a similar trend of PV values, where green tea treated Persian sturgeon revealed a lesser increase in PV vs. control during chilled storage.

### 3.7. Aerobic bacterial count (ABC)

One important reason why chilled stored shrimp quick spoilage occurs is spoilage bacteria, which causes bad odour, unattractiveness and makes shrimp not acceptable for consumption, and the ABC indicate the bacteriological load (Huss, 1988; Viji et al., 2015). ABC of the treated groups and control group during 28 days storage are showed in Table 5. On the 1<sup>st</sup> day, total bacterial count was in a range of log 4.30–4.47 cfu/g for all slots. During the storage, aerobic bacterial counts increased significantly ( $P < 0.05$ ). However, the trend was significantly lower ( $P < 0.05$ ) for the amla and green tea treated groups. At the 28th day of storage, the ABC of the control group raised to log 8.32 cfu/g, whereas, green tea treated sample showed a load of log 6.53 cfu/g and amla log 6.82 cfu/g (Table 5) which was significantly ( $P < 0.05$ ) lower vs. the control group. This log reduction showed a good correlation with sensory analysis score. The ABC support the chemical quality analyses (TVB-N, FFA and PV) well, and showed almost similar level of quality retardation in both treatment groups.

A previous study by Del Nobile et al. (2009) revealed that lemon extract and thymol in combination with modified atmospheric packaging (MAP) significantly ( $P < 0.05$ ) reduced the bacterial load vs. the control. ABCs revealed in the present study, indicates that green tea and amla have potential inhibitory effect on spoilage bacterial growth. This trend is in accordance with Ozogul et al. (2010) using sardine (*Sardinella gibbosa*) fillet treated with rosemary extract vs. the untreated group.

### 3.8. Effect of green tea and amla on melanosis formation of Indian white prawn at chilled storage

Melanosis score for Indian white prawn treated with green tea and amla extract and the control during 28 days of chilled storage study are showed in Fig. 7. On the 1st day, all samples displayed no melanosis, and no differences in appearances were revealed. During storage, melanosis formation showed an significant ( $P < 0.05$ ) increasing trend, and is in accordance to the finding of Nirmal & Benjakul (2011a, 2011b) showing that catechins in green tea have a potential effect on melanosis formation in Pacific white shrimp.

The present study noticed that, the green tea and amla treated groups remained appealing until 18th to 19<sup>th</sup> day and 16th to 18th day, respectively to the panellists as blackening was moderate (40–60%) and score obtained was within 4–6 range whereas, control reached a score

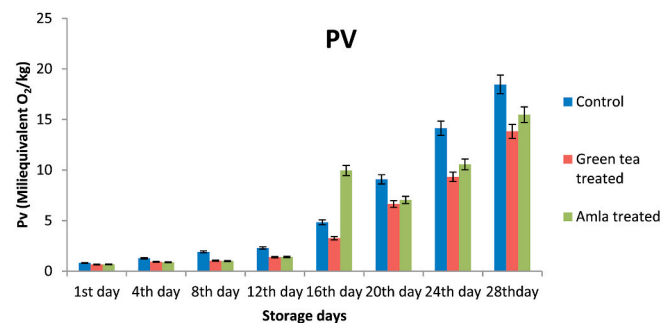
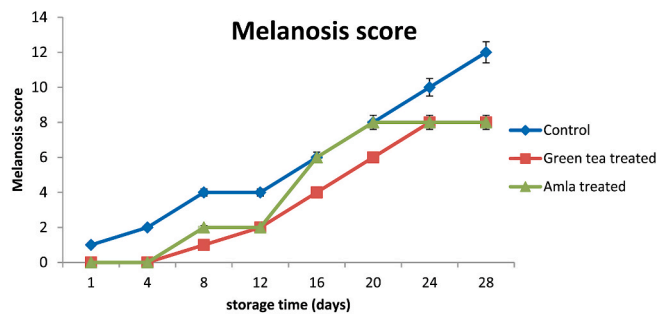


Fig. 6. Peroxide value (PV) of Indian white prawn treated with green tea – and amla extract, and control during 28 days of storage at 4 °C. Bars represent the standard error (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)





**Fig. 7.** Melanosis score of Indian white prawn treated with green tea – and amla extract, and control during 28 days of storage at 4 °C. Bars represent the standard error (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

above 8 which implies severe blackening ( $\geq 80\%$ ) from the 14th day and onwards ( $P < 0.05$ ). Therefore, an extract dose of 50 g/L of green tea and amla extract significantly ( $P < 0.05$ ) reduced melanosis of Indian white shrimp during chilled storage. This result is in accordance with the finding of Gokoglu and Yerlikaya (2008), where significant ( $P < 0.05$ ) inhibitory effect of grape seed treatment on melanosis formation in shrimp was revealed. The authors noticed that the treated group showed acceptable condition up to the 2nd day of storage, but the control group revealed a heavy blackening by the 3rd day of storage, and was considered as unacceptable by the sensory panel.

### 3.9. Effect of green tea and amla on sensory quality of Indian white prawn at chilled storage

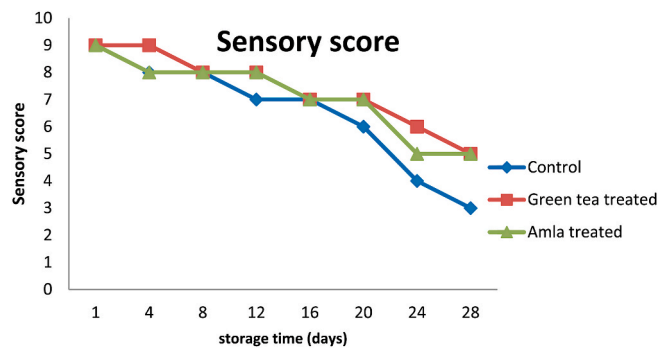
The changes in sensory qualities of Indian white prawn treated by green tea, amla and the control during chilled storage are showed in Fig. 8. On the 1<sup>st</sup> day, all samples had the score near 9 in the hedonic scale and the same likeness was observed in all treatments ( $P > 0.05$ ). During storage, the spoilage rate was significantly ( $P < 0.05$ ) faster for the control group. The sensory scores of green tea extract and amla extract treated lots were significantly repressed, compared to the control ( $P < 0.05$ ).

Generally, shelf life ends when essential sensory parameters such as off-odour and flavour become pungent or putrid due to bacterial activity (Huss, 1988) and even appearance of shrimp becomes unaccepted from consumer point of view. It is rejected, when it reach a score below 3–4 score in the hedonic scale. The present study revealed that, green tea and amla treatment was acceptable until 18th to 20th day and 17th to 19th day, respectively due to texture and odour. Appearance of treated lots was good to acceptable and score obtained was within 5–6 range whereas, control received score of below 4 before the treated groups ( $P < 0.05$ ). Thus, from the data indicated that the treated groups retained their good quality characteristics.

A study conducted by Fang et al. (2013) on Pacific white shrimp using pomegranate peel extract revealed that a decrease of sensory score was significantly inhibited in pomegranate peel extract treated sample compared to the control. In addition, Nirmal & Benjakul (2011a, 2011b) stated that Pacific white shrimp treated with lead seed showed higher score in likeness and quality compared to untreated group. However, sensory quality score varies from study to study due to initial species condition, dose differences and other environmental factors, but it can be suggested that natural extract inhibits the rapid loss of sensory score. The present study revealed that green tea and amla extract significantly impede the loss of sensory attributes in chilled stored Indian white prawn.

## 4. Conclusions

The increasing trend of biochemical indices viz. TBV, FFA, PV and



**Fig. 8.** Sensory score in hedonic scale of Indian white prawn treated with green tea – and amla extract and control during 28 days of storage at 4 °C. Bars represent the standard error (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

bacterial counts were significantly lower in treated Indian white prawn compared to the control. Treatment with green tea and amla extract can efficiently retard melanosis development and ameliorate the sensory quality of shrimp in chilled storage as overall acceptance of treated sample was encouraging compared to untreated group. Dip treatment in ethanol extract of green tea and amla enhance the storage quality and prolong the shelf life of Indian white prawn during chilled storage effectively. The present study also revealed that green tea and amla extracts are alternative natural preservative for fishery products instead of the abuse of synthetic additives.

Hence, treatment with green tea and amla extracts are suggested as an alternate aid to prevent overall quality loss in shrimp during post mortem handling and subsequent storage.

## Author's contributions

Aimen Firdous was involved in the concept design, data analysis and execution of this project. Preetham Elumalai was involved in Supervision, Conceptualization and acquisition of data's. Further improvement of manuscript by critical reviewing and finalization of the manuscript together with Einar Ringø.

## Declaration of competing interest

The authors have no competing interests. The research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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