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Total Polyphenol Content and Antimicrobial Activity of Commercially Available Tea and It's Use as a Fish Preservation

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ABSTRACT

Fish and fishery products being highly perishable and readily susceptible to chemical and microbial deterioration leads to reduction in the quality, wastage and economic loss. Maintenance of high-quality fish, so calls for adequate, effective and affordable preservative techniques of these nutritious resources. In the present study fish was treated with four different treatments namely Crude extract of green tea (GT_1), Concentrated extract of green tea (GT_2), Crude extract of black tea (BT_1) and Concentrated extract of black tea (BT_2). Tea is a very good source of polyphenols including bioactive chemicals, flavonoids, and catechins and their derivatives. The results of present research showed that highest (98.46±1.24 mg TAE g^{-1} dw) and lowest (49.60±1.95 mg TAE g^{-1} dw) phenolic content were found in aqueous solutions of GT_2 and BT_1 respectively. Total phenolic content in water extracts has shown in the following order GT_2>BT_2>GT_1>BT_1. Among the various tea extract tested, GT_2 exhibited greater antimicrobial activity followed by BT_2, GT_1 and BT_1.

Keywords: Fish preservation, Tea extract, Phenolics, Antimicrobial, Natural preservative

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INTRODUCTION

Fish and fishery products receive considerable attention as a good source of nutritional components in the human diet (Aubourg et al. 2007) andconsidered as an important source of quality animal protein in the human diet (Ozogul et al. 2006). Special attention has been paid to the long chain polyunsaturated fatty acids because of their multiple protective actions against human health problems like cardiovascular diseases. Presently India is the second largest fish producing and second largest aquaculture nation worldwide.

The fish production during 2015-16 was 10.79 million metric ton (MMT) with a contribution of 7.21 MMT from the inland sector and 3.58 MMT from the marine sector. Inland fisheries presently have a share of 66.81% in the total fish production of the country (FAO 2016).

The microbial safety of foods continues to be a major concern to consumers, regulatory agencies and food industries throughout the world. Many food preservation strategies have been used traditionally for the control of microbial spoilage in foods but the contamination of food and spoilage by microorganisms is a problem yet to be controlled adequately. Synthetic chemicals are often used as antimicrobials in food processing and storage to eliminate the foodborne pathogens, many of which contribute to increasing the resistance to antibiotics and having adverse side effects, even carcinogenic (Manea et al. 2014).

Tea is a very good source of polyphenols (10–30%, dry leaf weight) including bioactive chemicals, flavonoids and catechins and their derivatives. Tea polyphenols are also known for their antibacterial activity. In general, antibacterial activity may decrease withthe increase oftea fermentation extent, green teaimplyiesstronger activity than black tea (Chan et al. 2011). Till date, antimicrobial activity of tea has been studied and found that tea is containing some active components like polyphenols which hasantimicrobial effect against various microorganisms. These compounds include epicatechin, catechin, caffeine, chlorogenic acid, gallic acid, theobromine, theophylline, gallocatechin, epigallocatechin gallate, catechin gallate, epicatechin gallate. Theaflavingreen tea is rich in polyphenols, mainly catechins and catechin derivatives including epigallocatechin-3-gallate (EGCG), epicatechin(EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and gallocatechin gallate (GCG) (Tsai et al. 2013). Studies have reported that epigallocatechingallate (EGCG) available in green tea adds to antibacterial activity (Almajano et al. 2008). Furthermore, EGCG has been reported to be capable of inhibiting the growth of pathogenic and spoilage microbialsuch asBacillus, Campylobacter, Clostridium, *E. coli,* L. monocytogenes, Pseudomonas, Staphylococcus aureus, Salmonella andVibrio (Xi et al. 2012). Thus the objective of present study is to determine the total polyphenol content and antimicrobial activity of commercially available tea and their use as a fish preservative.

Scientific classification of tea

Kingdom : Plantae
Order : Ericales
Family : Theaceae
Genus : Camellia
Species : C. sinensis
Binomial name : Camellia sinensis (L.) Kuntze

MATERIALS AND METHODS

Green and black tea extracts preparation

Crude aqueous infusions were made by adding 2 g of leaf to 100 ml boiling water, allowing the suspension to stand for 10 min and removing solid matter by filtration. The extract was then concentrated to one-fifth of the initial volume following the method described by Tiwari et al.(2005). Only fresh extracts were used for this work, as changes of chemical composition of tea occur in the storage(Yam et al.1997). The study was conducted as a Completely Randomized Design, with four treatments. The treatments were Crude extract of green tea (GT_1), Concentrated extract of green tea (GT_2), Crude extract of black tea (BT_1) and Concentrated extract of black tea (BT_2). The experiments were done in triplicate.

Determination of total phenolics

Total phenolics were determined by a colorimetric method of Folin-Ciocalteu reagent (Singh et al. 2002). The green tea and black tea extract were dissolved in 80% aqueous methanol (2:1 v/v). 0.5 ml of the solution was well mixed with 1 ml of diluted Folin-Ciocalteu reagent (1:10 with distilled water) and 0.8 ml of 7.5 % Sodium carbonate. The mixture was allowed to stand for 30 min at room temperature and their absorbance was measured at 765 nm with a spectrophotometer (Lightwave S2000 UV/VIS diode array spectrophotometer). The standard curve was prepared using 10, 20, 30, 40, 50, 60, 70 and 80 mgL^{-1} solutions of tannic acid in methanol: Water (80:20 v/v). Total phenol values were expressed in

tannic acid equivalent (TAE) (mg g^{-1} of dry mass for green tea). The estimation of phenolic content was replicated five times, and the results expressed as mean ± SD (standard deviation).

Determination of antimicrobial activity

The antimicrobial activity was measured using a well diffusion method accordingto the National Committee for Clinical Laboratory Standard (NCLSI 2011). In this method, petriplates containing approximately 25-30 ml of Plate Count Agar medium were inoculatedusing a cotton swab with culture of the bacterial strains. Wells (10 mm diameter) werepunched in the agar and filled with 100 µl of tea extracts (green and black) and allowed for incubation at 37°C for 24 hours. Each treatment was done in triplicates. The average of three replicates for each treatment (green and black tea extracts) wascalculated and antimicrobial activity (mm) was assessed by measuring the diameter of inhibition zone around the well.

Statistical analysis

All the data were checked for normal distribution with normality plots prior toanalysis of variance (ANOVA) to determine significant differences among means at α =0.05 level, using statistical tools of Microsoft Office Excel (2007).

RESULTS AND DISCUSSION

Total phenolic content of green and black tea extracts

The primary objective of this study was to determine the total phenolic content of green and black tea extracts (GT1, GT2, BT1, BT2) in terms of tannic acid equivalent (the standard curve equation: $y = 0.0275x + 0.0083, R^2 = 0.985$) is shown in Fig. 1. Antibacterial activity is generally governed by total phenolics, flavanoids and antioxidant ability of an extract (Gopal et al. 2016). In the present study, the highest (98.46±1.24 mg TAE g^{-1} dw) and lowest (49.60±1.95 mg TAE g^{-1} dw) content were found in aqueous solutions of GT2 and BT1 respectively. Total phenolic content in water extracts decreased in the following order GT2>BT2>GT1>BT1.

The results of the present study is in conformity with the findings of Samman et al. (2001), Rababah et al. (2004), El-Hanafy et al. (2011), Gai et al. (2014), FĂRCAŞ et al. (2015), Nibiret al. (2017). El-Hanafy et al. (2011) reported that the total phenolic content of 2% green tea extract (GTE) was 57.7 mg g^{-1} gallic acid equivalent whereas Gai et al. (2014) found 96.6 mg gallic acid equivalents (GAE) per 100 g of product.

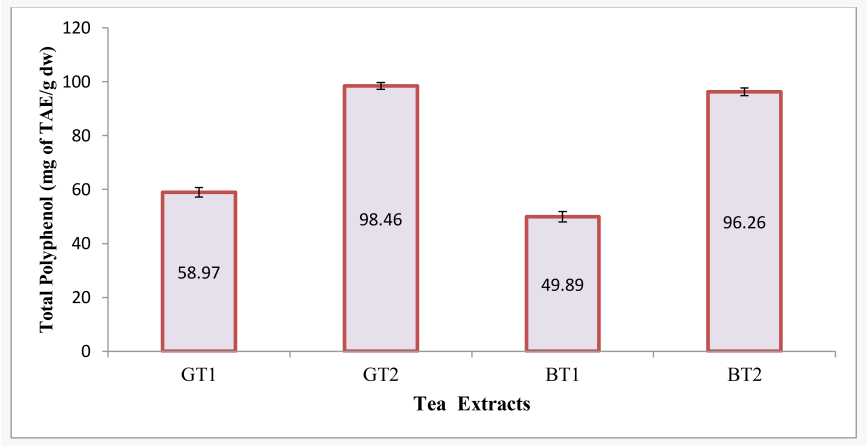

Figure 1. Total phenolic content of green tea and black tea extracts.

On the other hand, Samman et al. (2001) reported the total phenolic compounds of green tea extracts, as 117.3 mg gallic acid equivalents/g which as similar to thereport of Yerlikaya and Gokoglu(2010) i.e. 126±4.5 mg g^{-1} tannic acid equivalent. However, according to Shinde et al. (2015), the phenolic content of green tea extract (GTE) was observed to be 212±20.55 mg g-1 tannic acid equivalentwhich is evident to be higher than the results reported in present study.

Farcaset al. (2015) reported that the concentration of phenolic compounds in green and black tea were 45.90 mg GAE/100 ml and 39.60 mg GAE/100 ml respectively which wasvery similarto the result of Rababah et al. (2004) i.e. 59.8mg CAE/g forgreen tea extract and 59.3 mg CAE/g for black tea extracts. According to Nibir et al. (2017), green tea contained significantly ($p< 0.05$) higher amount of total phenolic and flavonoid compounds compared to other tea varieties. They reported that the total phenolic content of green tea extract and red dust tea to be 26.33 ± 1.73 and 8.20 ± 0.49 mg g-1 gallic acid equivalent respectively, which is lower than the values recorded in the present study.

The level of phenolics in the black tea extracts prepared from a single species of Taiwanese tea (TTES No.12) was found to be 74.76 ± 2.66 mg/g (Lin and Lin 2005) which is lower than the phenolic contents determined in the present study. Higher values werereported by Lee et al. (2002) wherein the total phenols equal to 124 mg gallic acid equivalents/g was measured in the case of black tea. The brewing condition such as extraction temperature, period of extraction, ratio of tea leaves to extracting water, and stirring are important factors for determining the total phenolic content in green tea extract (Liebertet al. 1999). Rusak et al. (2008) also reported that the total content of polyphenols in tea extracts correlates with extraction time. The variation in the polyphenolic composition of the different tea products is ascribed to the different process appliedparticularly the leaf maceration and autooxidation steps. Several other factors have been identified influencing the polyphenol content of a tea product. These include genotype, geographical origin, soil composition, harvesting time, postharvest treatment and physical structure of the leaves (Lin et al. 2000).

Antimicrobial activity of green tea and black tea extracts

The secondary objective of this study was to determine the antimicrobial activity of Camellia sinensis leaf extract. The antibacterial activity was determined by agar well diffusion method (NCLSI 2011) and results are presented in Fig. 2. The figure revealed the antimicrobial properties in zone of inhibitions or halos

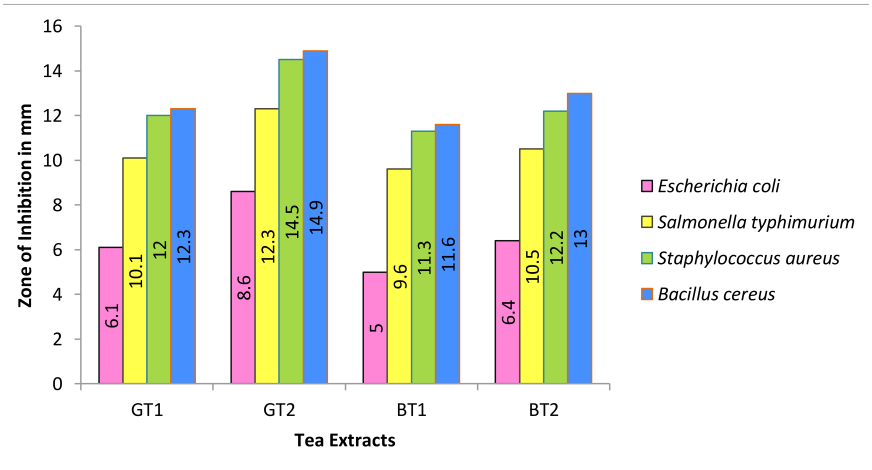

Figure 2. Antimicrobial activity of green tea and black tea extracs against some food pathogens.

(mm) that was exhibited by four different strains of bacteria when treated with this leaf extract. Among the extracts, GT_2 displayed the largest average halo against Bacillus cereus (14.9 mm) and the smallest average halo against Escherichia coli (5.0 mm) was given by BT_1.

The results revealed that all of the tested bacteria were susceptible to the exposure of all four kinds of tea extracts. Among the varieties of tea extract tested, GT_2 exhibited greater antimicrobial activity followed by BT_2, GT_1 and BT_1. The green tea extract was more effective as compared with black tea extracts to inhibit bacterial pathogens. The decreased antibacterial activity of black tea is probably due to fermentation. Extracts from all the teas studied had an inhibitory effect against the tested strains.

The results of the present study is supported by the findings of Nibir et al. (2017),Almajanoet al. (2008) who reported green tea extract was much more effective to inhibit bacterial pathogens than all other black tea extracts and their experimental result showed almost similar zone of inhibitions for the ascribed bacteria as found in this study. The decreased antibacterial activity of black tea is probably due to fermentation. Relativelylower levels of polyphenols in black tea can be attributed to the conversion of tea polyphenols into theaflavin and thearubigins during fermentation process (Nibir et al. 2017). A similar result was also reported by Üstündağ et al. (2016) who studied antimicrobial activity of black tea and reported that the antimicrobial activity depends on types microorganism, variety of tea used, composition and content of the phenolics present inextracts and process of solvent extraction.

Antibacterial activity of green and black tea extracts has been attributed to the presence of catechins (importantly EGCG and ECG). Most polyphenols show antimicrobial activity like catechins, the polyphenols in tea, proanthocyanidins and hydrolysable tannins.The tolerance of bacteria to polyphenols depends on the bacterial species and the polyphenol structure (Almajanoet al. 2008, Nibiret al. 2017). In general, gram negative bacteria are more resistant to polyphenols than gram positive bacteria, perhaps due to the difference in cell wall compositions (Negi et al. 2003). There are various hypotheses that explain the mechanism of action of C. sinensis although, the exact mode of action is still unclear. However, damage of bacterial cell membrane, inhibition of crucial enzyme activity, fatty acid synthesis and various other physiological activities may contribute individually or synergistically to the overall antimicrobial activity of the tea extracts. The zoneinhibitiondepends on the bacterial strain, the kind of extract and the concentration of extract (Almajanoet al. 2008,Nibiret al. 2017) prepared from tea.

CONCLUSION

The total phenolic content of green and black tea extracts was observed to be vary between 98.46±1.24 to 49.60±1.95 mg TAE g^{-1} dw (dry weight) indicating that the phenolic content is affected by the concentration and type of tea used. This event suggests the possibility of utilizing crude aqueous tea leaves extract as a source of antimicrobial and antioxidants.

The antimicrobial activity of crude aqueous green and black tea leaf extracts (GT_1, GT_2, BT_1, BT_2) was tested on four bacterial strains (Bacillus cereus, Escherichia coli, Salmonella typhimurium and Staphylococcus aureus) where GT_2 was more effective against all the tested strains and BT_1 was found to be least effective. The findingsof the present study report thatCamellia sinensis is a promising and potential natural preservative because of its wide range of inhibition against both gram positive and gram negative bacteria. Thus, it may be concluded that the phenolic content of tea extract prepared varied with the concentration of tea leaves used and the variety i.e. green tea and black tea. The processors therefore have the possibility to choose between green and black tea as well as concentration of tea as a source of antioxidant and antimicrobial agentaccording to their convenience-based on availability and cost. However, GT_2 had highest total phenolic content and exhibited greater antimicrobial activity as compare to other treatments.

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