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SYNERGISTIC EFFECT OF PINEAPPLE PEELS & GREEN TEA ON ANTIOXIDANT LEVELS OF PINEAPPLE PEELS INFUSED KANGRA GREEN TEA

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ABSTRACT
There is growing interest of use of the plant-derived raw materials such as fruit peels obtained from domestic and industrial sources as natural source of antioxidants. Tea is one of the most consumed beverages in the world, after water. It is recognized as a dietary source of polyphenolic compounds, such as catechins, theaflavins, flavonol glycosides, flavone glycosides, caffeine, gallic acid, and proanthocyanidins. The aim of this study was to evaluate the content of bioactive compounds and antioxidant activity of pineapple fruit peels infused green tea using Folin-Ciocalteu method and ferric-reducing antioxidant power. The total phenolic content was significantly higher in case of green tea infused with pineapple peels (63.46 ± 0.05 mg TAE/g) ($p \leq 0.05$) as compared to green tea (62.93 ± 0.07 mg TAE/g) and pineapple peels (49.33 ± 0.04 mg TAE/g). The pineapple peels infused green tea contains even higher antioxidant levels (18.93 ± 0.05 mg AAE/ g) than pineapple peels (15.93 ± 0.04 mg AAE/ g) and green tea (16.13 ± 0.07 mg AAE/ g) as well.

KEYWORDS: pineapple, Kangra green tea, pineapples, antioxidant levels.

INTRODUCTION

In India, fruit production and consumption has increased due to economic growth and also due to increased awareness about the vital fruit nutrients content and secondary phytochemical compounds (Indian Horticulture Database, 2013). Pineapple is a tropical fruit popular throughout the world. It is rich in the antioxidants namely flavonoids, vitamin A and C. After pineapple processing, many thousands of tons of peel are produced as an agricultural by product which are not being utilized judiciously leading to a waste management problem. The pineapple peel is well known ingredients in ethnomedicine and could be a potential source for the extraction of beneficial bioactive compounds (Alejandra *et al*., 2011). Natural antioxidants present in pineapple peels have gained increasing interest as various epidemiological studies have found their association with a lower risk of cardiovascular disease, diabetes, obesity, neurodegenerative, gastrointestinal disorders and cancer. The functional compounds present in pineapple peels are vitamins, polyphenolic compounds such as gallic acid, epicatechin, catechin, ferulic acid and carotenoids (Li *et al*., 2014)

Green tea (*Camellia sinensis, Theaceae*) is the most widely consumed beverage, because of attractive aroma and special taste. The Kangra green tea has higher TPC and antioxidant levels than Darjeeling green tea but slightly bitter in taste (Kaur *et al*., 2015). The health-promoting effects of green tea are mainly attributed to its polyphenol content particularly flavanols and flavonols, which represent 30% of fresh leaf dry weight (Piljac-Zegarac *et al.*, 2010). The chemical composition of green tea is complex: proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-*N*-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, and sucrose; and trace amounts of lipids (linoleic and α-linolenic acids), sterols (stigmasterol), vitamins (B, C, E), *etc* (Fu *et al.*, 2011). Pekal *et al.* (2011) have reported antioxidants levels in commercial available flavoured black tea and found that commercially available pineapple flavoured black tea contain significant content of main catechins, particularly the concentration of EGCG as compared to commercial available black tea. However, there is paucity of literature regarding the infusion of Indian green tea with fruit peels. The present study has been managed to evaluate the various biochemical properties like phenolic levels, antioxidative capacity and ascorbic acid content in case of green tea infused with pineapple peels, which itself is a rich source of all considered properties.

MATERIALS & METHODS

Processing of Fruit peels

Peels of pineapple were collected separately from local market and dried in hot air oven at 70 degrees for 3-5 days. The dried fruit peels were powered and stored at room temperature.

Sample extraction

A sample of 1 g green tea containing 25% (250 gm) of dried pineapple peels was weighted (±0.001 g) into a beaker and 100 ml of boiling distilled water was added. After brewing for 5 min the blend was removed and the

extract was cooled down. All analyses of aqueous tea extracts were done in triplicate.

Determination of total phenolics

Total phenols concentrations were measured using colorimetric Folin-Ciocalteu method. Briefly, 100µL of tea infusion were mixed with 2ml of sodium carbonate (2%), 2.8mL deionized water and 100µL of Folin–Ciocalteu reagent (50%). After incubation at room temperature for 30 min, the absorbance was read at 760 nm on a UV–Vis spectrophotometer. Concentration of total phenols was calculated using a standard curve of aqueous solutions of tannic acid and expressed as mg tannic acid equivalents (TAE) g^{-1}. All measurements were taken in triplicate.

Ferric Reducing Antioxidant Activity (FRAP)

The FRAP assay is a precise and common method for measurement of antioxidant capacity of tea infused fruit peels. To 2.5 ml of the tea infused extract added 2.5 ml phosphate buffer (0.2 M, pH-6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then boiled at 50°C for 20 minutes, then rapidly cool, mixed with 2.5 ml of 10% trichloro acetic acid and centrifuge at 3000 g for 10 minutes. The supernatant was collected and 2.5ml of distilled water was added and 0.5 ml of 0.1% ferric chloride mixed well and allowed to stand for 10 minutes. The increase in the absorbance at λ 700 nm is used to measure the reducing power. The antioxidants in samples were derived from a standard curve of ascorbic acid ranging from 10 to 100 µg/mL. The total antioxidant power was expressed as mg ascorbic acid equivalent (AAE)/ g.

Ascorbic acid

The 2, 4- dinitrophenylhydrazine (DNP) method is used in present study to determine the ascorbic acid levels in which reduced ascorbic acid is oxidised and dehydroascorbic acid, followed by coupling with 2,4-dinitrophenylhydrazine under controlled conditions to give red coloured osazones (Roe and Keuther, 1943). To 0.6 ml of tea infusions, distilled water was added to make volume up to 3ml. Then, 1ml of 2, 4-DNP was added to each tube followed by incubation at 37°C for 3 hours. 7ml of 80% H_2SO_4 was then added to each tube in order to completely dissolve the red osazones crystals. Total amount of ascorbic acid was calculated using standard curve of ascorbic acid at wavelength of 540nm.

Statistical analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by student's t-test ($p < 0.05$).

RESULTS & DISCUSSION

It is anticipated that fruit waste material especially peels, which are considered as vital source of natural antioxidants can be utilised in food industries to enhance the nutritional value of beverages and food of daily consumption, like green tea.

The present study was conducted on pineapple peels, green tea, and pineapple infused green tea to evaluate vitamin C content which ranged from 9.05 mg/g ± 0.03 to 11.63 mg/g ±0.07). However, significantly higher amount of ascorbic acid was found in case of green tea infused with pineapple peels (11.63 ±0.07 mg/g) as compared to green tea (10.79 ±0.05 mg/g) and pineapple peels (9.05 ± 0.03 mg/g). Ascorbic acid present in high amount in fruit peels is required in the synthesis of collagen in connective tissues, neurotransmitters, steroid hormones, carnitine, and conversion of cholesterol to bile acid and enhances iron bio-availability (Robert *et al*., 2000). Pineapples peels have significant amount of ascorbic acid as reported by Kongsuwan *et al*., (2009). It has been reported that ascorbic acid present in fruits protects some flavonoids, such as anthocyanins present in green tea against oxidative degradation. Vermeer *et al*. (1999) reported that intake of ascorbic acid with green tea consumption to have inhibiting effects on endogenous formation of N-nitroso compounds. Aleksandropoulo *et al*. (2006) reported that addition of ascorbic acid increases the antioxidative levels of green tea.

There was significant amount of phenolic content in pineapple peels, green tea, green tea infused with pineapple peels as reported by Li *et al*. (2014). Total phenolic content was significantly higher in case of green tea infused with pineapple peels (63.46 ±0.05 mg TAE/g) ($p \leq 0.05$) as compared to green tea (62.93 ±0.07 mg TAE/g) and pineapple peels (49.33 ±0.04 mg TAE/g) as shown in Fig. 1. Polyphenols from both green tea and pineapple peels might have exhibited synergistic effect which thereby resulted in enhancing the phenolics in pineapple infused tea, Gallic acid, catechin, epicatechin and ferulic acid were found to be the main polyphenolics in pineapple peels (Uchoi *et al*., 2017).

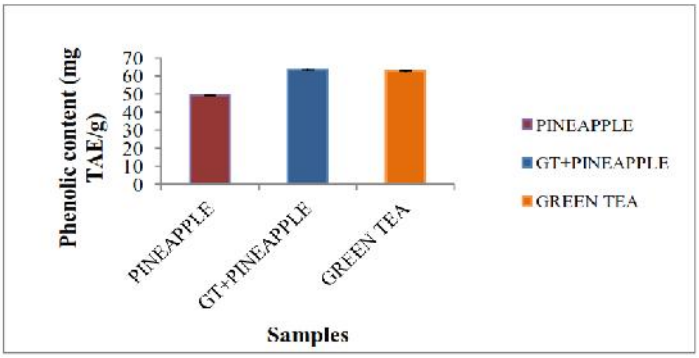

FIGURE 1: The figure depicts the levels of phenolics in pineapple peels, pineapple peels infused green tea and green tea respectively

Moreover, high content of Myricetin, a polyphenol present in the pineapple shell is also responsible for its high antioxidant capacity (Larrauri *et al*., 1997). The primary catechins found in green tea are epicatechin (EC), epigallocatechin (EGC), epicatechin-3 gallate (ECG), and epigallocatechin-3-gallate (EGCG), the most potent one. The increased phenolic content in pineapple infused green tea could be due to additive effects due to interactions of hydroxyl group of ferulic acid found in pineapple with epicatechin or gallic acid found in pineapple and green tea (Li *et al*., 2014).

The present study reported high content of antioxidative capacity in pineapple peels (15.93 ±0.04 mg AAE/ g) as shown in fig. 2. Pineapple peels contain good amount of natural antioxidants present in the form of phenols, flavonoids and ascorbic acid, β-carotene and other bioactive compounds such as gallic acid, catechin, epicatechin, and ferulic acid which contribute to the antioxidant activity in an synergistic and antagonistic effect (Li *et al*., 2014; Ravimannan and Nisansala, 2017). The pineapple peels infused green tea contains even higher antioxidant levels (18.93 ±0.05 mg AAE/g) than pineapple peels and green tea (16.13 ±0.07 mg AAE/ g) as well. The green tea has strong antioxidant capacity due of the presence of various bioactive compounds such as catechins (90%), oxyaromatic acids, tannins, flavonols, thearubigins, theaflavins, etc (Hajimahmoodi *et al*., 2008). The synergistic effect of various bioactive compounds present in green tea and pineapple peels might have enhanced the levels of antioxidants in pineapple infused green tea.

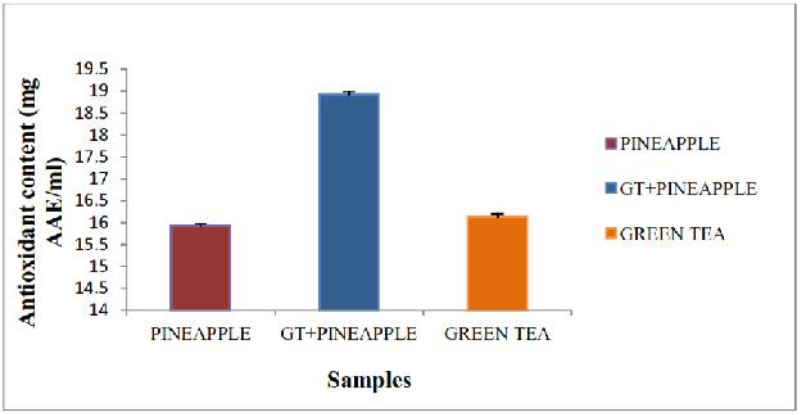

FIGURE 2: The bar chart depicts the antioxidant content in pineapple peels, pineapple peels infused green tea and green tea, respectively

Rapid increase in demand & consumption of pineapple (a tropical fruit) worldwide in processed form is the major cause of massive waste generation (40-80%). Pineapple peels are packed with extensive amount of phenolic compounds, flavonoids and ascorbic acids along with rich source of vitamins A, B and C. Green tea itself is also the main source of antioxidants and polyphenols. Infusion of pineapple peels in green tea could be employed in production of new natural antioxidant beverage infused green tea which will not only boost up the sustenance value but will also improve the taste and aroma of green tea.

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