



EVALUATION OF INDIAN KANGRA GREEN TEA (*CAMELLIA SINENSIS* (L) *O KUNTZE*) INFUSED WITH PEELS OF LOCAL FRUITS (POMEGRANATE, LEMON, MOSAMBI, ORANGE AND BANANA) AS A NATURAL SOURCE OF ANTIOXIDANTS

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ABSTRACT

In India, with the pace of economic growth, consumption of fruits in various forms has increased many folds. However, the optimum use of fruit waste, especially fruit peels which are a rich source of bioactive compounds, is still not fully explored. Fruit peels contains high amount of phenolics and flavonoids and strong natural antioxidant compounds required for the prevention of various lifestyle disorders e.g., diabetes, cardiovascular disease, etc. The Kangra green tea available in North India, has high antioxidant levels. The present study was conducted with an aim to estimate the levels of protein, tannins, phenolics and flavonoids content and antioxidant levels of Kangra green tea infused with peels of locally available fruits (pomegranate, lemon, mosambi, orange and banana). The highest protein levels were found in pomegranate peels infused green tea (78.35 ± 3.16 mg/ml) followed by citrus peels infused green tea (47.50 ± 0.360 mg/ml to 52.39 ± 0.21 mg/ml) and least in banana infused green tea (46.25 ± 2.10 mg/ml). The total phenolic content (TPC) of green tea infusion with fruit peels is pomegranate (51.33 mg TAE/g) > lemon (33.73 mg TAE/g) > mosambi (31.38 mg TAE/g) > orange (8.93 mg TAE/g) > banana (6.13 mg TAE/g). Test for antioxidant level revealed that Kangra green tea infused with pomegranate peels (15.53 mg AAE/g) ($p \leq 0.05$) has significantly higher amount of antioxidant levels as compared to other fruit peels infused green tea (orange, lemon, mosambi and banana). The present study concluded that the peels of locally available fruits can be used to enhance the antioxidant properties, nutrition and taste of green tea in a cost-effective manner so as to lower the risk of various lifestyle diseases such as diabetes, obesity and cardiovascular diseases, etc.

Keywords: Kangra green tea, Fruit peels, India, Antioxidant levels.

1. INTRODUCTION

India is the second largest producer of green tea in the world after China which includes the famous Assam tea, Darjeeling tea, Nilgiri tea and Kangra tea. The major tea producing states in India are Assam, Himachal Pradesh, West Bengal, Tamil Nadu, Kerala and Karnataka. In Asia, daily around 1.2 litre of green tea is consumed by a person which provides high levels of polyphenols and other antioxidants [1].

Kangra green Tea (*Camellia sinensis* (L) *O Kuntze*) is a rich source of alkaloids such as caffeine, theobromine, and theophylline which provide stimulant effects. L-theanine, an amino acid compound found in green tea has calming effects on the nervous system. Green tea also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as chromium, manganese, selenium or zinc, and certain phytochemical com-

pounds. The various flavonoids present in Kangra green tea (flavan-3-ols) are catechins (C), (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin (EC) [2]. Catechin and EGCG are the stronger catechins among the group of five catechins in Indian green tea (Kangra, Nilgiri, Assam, and Darjeeling). Green tea contains various phenolics such as gallic acid, vanillin, myricetin, rutin, protocatechuic acid, quercetin and kaempferol. However, in Indian green tea, Vanillin was found to be a predominant phenolic acid followed by gallic acid and myricetin where as rutin, kaempferol, quercetin and protocatechuic acid was not detected [3]. The Kangra green tea has higher total phenol content (TPC) and antioxidant levels than Darjeeling green tea [4]. India is the second largest producer of fruits after China, with a production of 81,285 million tonnes of fruits

from an area of 6,892 million hectares. Various fruits are grown in India, of which banana (32.6%), mango (22.1%), citrus (12.4%) and papaya (6.6%) are the major ones [5]. The fruits peels represent almost 30% of the total weight and in the primary by-product of fruit processing units. The peels are a source of bioactive compounds such as phenolics, flavonoids, ellagitannins, proanthocyanidin compounds, minerals, and complex polysaccharides. Thus, peels can be considered as a good source of antioxidants for foods and functional foods against cancer, heart diseases and lifestyle diseases. Pomegranate peels have around 124 different phytochemicals and nearly 48 phenolic compounds. The citrus peels have flavonoids such as, hesperidin and naringin and another class of O- methylated glycones of flavones. The peels of banana fruit contain various antioxidant compounds such as galocatechin and dopamine [6].

The peels of various fruits such as pomegranate, orange, mausami, lemon have strong antioxidant activity. The peels also have anticarcinogenic and anti-inflammatory properties which suggest its possible role in the prevention and treatment of several types of cancer and cardiovascular disease [7].

In India, green tea consumption is rising at a rate of more than 10 percent annually. Fruit infused green teas are gaining popularity not only because of their fragrance, taste and lower amounts of caffeine but also due to high amount of natural antioxidants. It was reported that dried fruits (quince, cranberries, orange, lemon, and grapefruit) infused green tea have sensory characteristic of the blends (taste and aroma) as well as antioxidant properties [8]. Since, there are no studies done on Indian Kangra green tea supplemented with peels of various locally available fruits, the present study was undertaken to evaluate the protein, tannins, phenolics and flavonoid content and antioxidant levels of fruit peel infused Kangra green tea.

2. MATERIAL AND METHOD

2.1. Processing of Fruit peels

Fruit peels of banana, orange, lemon, mosambi and pomegranate were collected separately from local market and dried in hot air oven at 70 degrees for 3-5 days. The dried fruit peels were powdered and stored at room temperature.

2.2. Sample extraction

One g green tea containing 25% (250gm) of dried banana, orange, lemon, mosambi and pomegranate

peels was weighted 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. All analyses of aqueous tea extracts were done in triplicate.

2.3. Estimation of protein content

The protein concentration in the fruit peel extract was done by the Lowry protein assay method [9].

2.4. Determination of tannin Content

The tannins were determined by Folin-Ciocalteu method [9]. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% Na_2CO_3 solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of ascorbic acid (20, 40, 60, 80 and 100 $\mu\text{g}/\text{ml}$) were prepared. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of AAE/g of extract.

2.5. Estimation of total phenol content (TPC)

The total phenol content (TPC) was determined by spectrophotometer using tannic acid as standard with some modifications [10]. 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of 1/10 dilution of Folin-Ciocalteu's reagent in water. 4.0 ml of a sodium carbonate solution (7.5% w/v) was then added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of tannic acid equivalent per g dry weight (mg TAE/g).

2.6. Determination of Total flavonoid content

Total flavonoid content was measured by the modified aluminium chloride colorimetric assay [11]. The reaction mixture consisted of 1 ml of extract and 4 ml of distilled water taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was added and after 5 minutes, 0.3 ml of 10% aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was added and final volume of the mixture was brought to 10 mL with double-distilled water. The absorbance for test and standard solutions were

determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve and was expressed as mg Ascorbic acid equivalent AAE/g of extract.

2.7. Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant capacity was determined spectrophotometry, using ascorbic acid as standard using the modified FRAP assay [12]. 0.1 ml of extract was taken and to it, 0.9 ml of ethanol, 5 ml of distilled water, 1.5 ml of HCl, 1.5 ml of potassium ferricyanide, 0.5 ml of 1% SDS and 0.5 ml of 0.2% of ferric chloride was added. This mixture was boiled in water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 750 nm to measure the reducing power of the tea extract. The antioxidants in samples were derived from a standard curve of ascorbic acid and were expressed as mg ascorbic acid equivalent (AAE)/ g.

2.8. 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 one way ANOVA ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

Agro-industrial wastes such as fruit peels are good source of phenolic compounds [13]. Green tea with fruit peels can be used as tasty beverage enriched with natural antioxidant due to presence of vitamin A, B6, C, E, polyphenol (Xavonoid, Xavanol, Xavonol, iso Xavone, quercetin, catechin, epicatechin, etc.), co-enzyme Q10, carotenoid, selenium, zinc and phytochemical compounds [14].

The protein levels of various fruit peels infused green tea are shown in Table 1. The protein levels in fruit peels infused green tea ranged from 47.50±0.36 mg/ml to 78.35±3.16 mg/ml. The highest protein levels were found in pomegranate infused green tea (78.35±3.16 mg/ml) followed by citrus infused green tea (47.50±0.360 mg/ml to 52.39±0.02 mg/ml) and least in banana peels infused green tea (49.23±2.10 mg/ml). The green tea with pomegranate and lemon peels had significantly higher protein level ($p \leq 0.05$) as compared to green tea (41.47±0.452mg/ml) as well as other fruit peels infused tea (orange, mosambi, and banana). It was

reported that pomegranate fruits peels powder (PPP) contained higher content of lysine, leucine, aromatic fatty acids (phenylalanine and tyrosine), threonine and valine [15]. The protein levels in all citrus infused green tea were similar (orange, mosambi and lemon) but higher than Kangra green tea. The additive effect of presence of L-theanine from Kangra green tea which has tranquilizing effects in the brain along with proteins from the fruit peels could have great economic value making it a good anti-stress beverage.

The highest amount of tannins was found in green tea with pomegranate (83.38±3.93mg AAE/g) followed by banana infused green tea (74.55±6.57mg AAE/g) and least was found in citrus peels infused green tea (orange, mosambi and lemon) (43.83±2.10 mg AAE/g to 61.11±6.3mg AAE/g) (table 1). The amount of tannins in pomegranate and banana infused green tea was significantly higher ($p \leq 0.05$) than citrus fruit peels infused green tea (orange, mosambi and lemon) and green tea. This could be due to synergistic effect of hydrolysable tannins present in pomegranate peels mainly punicalin, pedunculagin, and punicalagin as well as tannins present in green tea such as epigallocatechingallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG), and epicatechin (EC). Ellagitannin is tannin present in pomegranate peels which break down into hydroxybenzoic acid such as ellagic acid which has strong antioxidant activity.

The Kangra green tea with pomegranate (51.33±2.82 mg TAE/g), lemon (33.73±1.69 mg TAE/g), mosambi peels (31.38±3.5 mg TAE/g) had significantly higher amount of phenolics as compared to Kangra green tea (23.49±1.64 mg TAE/g) ($p \leq 0.05$) (Fig. 1). The various phenolics present in fruit peels are gallic acid and chlorogenic acid [15]. In citrus fruits peels, ferulic and sinapic acids are found in high amounts [16]. In the present study, the levels of flavonoid were similar in all fruit peels infused green tea except in orange peels infused green tea which had slightly higher flavonoids (50.16±1.97 mg AAE/g) (Table 1). The various flavonoids present in fruit peels are anthocyanins, flavan 3-ols, and flavonols. Pomegranate peels contain abundant quantity of phenolics and flavonoids and their polyherbal combination with green tea flavonoids such as quercetin derivatives provide a bioactive mechanism to reduce free radical-induced oxidative stress [17]. These pomegranate flavonoids show antioxidant activity with indirect inhibition of inflammatory markers such as tumor necrosis factor-alpha (TNF- α) [18]. The citrus

fruit peels contain glycosidic flavanones such as naringin and hesperidin which might increase the reducing

power of the green tea resulting in high chelating iron activity [17].

Table 1: The figure depicts protein, tannins, flavonoid levels in Fruit peels infused green tea (banana, orange, lemon, pomegranate and mosambi) and green tea.* denotes significance at $p \leq 0.05$ as compared to green tea

Samples	Protein (mg/ml)	Tannins (mg AAE/g)	Flavonoids (mg AAE/g)
Green tea	41.47±0.452	49.25±6.33	44.66±5.65
Banana + GT	46.25±2.10	74.55±6.57* (p=0.0007)	45.16±0.63
Orange + GT	48.507±3.16	43.83±2.10	50.16±1.97
Lemon + GT	52.39±0.02* (p=0.0003)	61.11±6.33	42.66±1.41
Pomegranate + GT	78.35±0.16* (p=0.0001)	83.38±3.93* (p=0.0001)	43.16±3.53
Mosambi + GT	47.50±0.36	44.77±4.00	43.25±3.40

* Denotes significance at $p \leq 0.05$ as compared to green tea

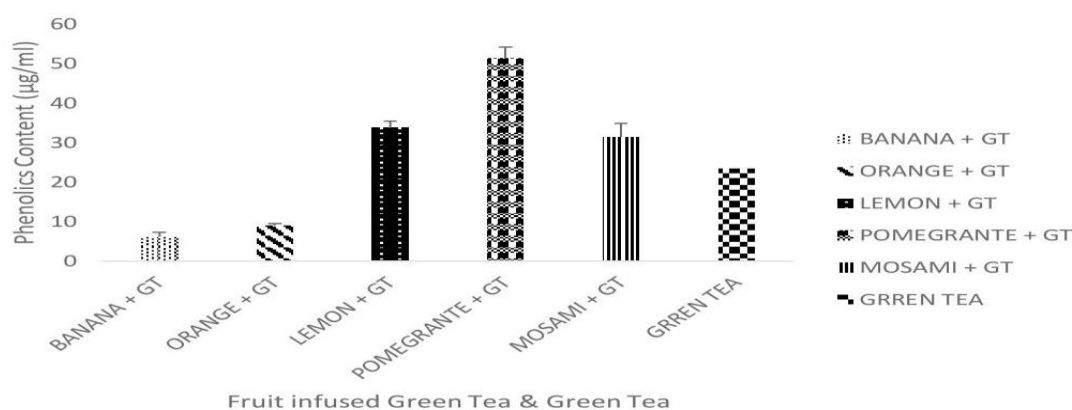


Fig. 1: The bar graph showing the phenolic levels in fruit peels infused green tea (banana, orange, lemon, mosambi and pomegranate) and green tea in mg TAE/ g

The high antioxidant activity was observed in green tea with pomegranate peels (15.53 ± 0.56 mg AAE/g) followed by lemon (14.83 ± 0.42 mg AAE/g), banana (11.03 ± 0.141 mg AAE/g), orange (10.13 ± 0.49 mg AAE/g) and mosambi peels (9.83 ± 0.28 mg AAE/g) infused green tea. The antioxidant levels in pomegranate peels infused tea are induced through ellagitannins and hydrosable tannins and punicalagin [19]. The green tea with pomegranate peels can be used as anti-diabetic beverage as it decreases glycemic index through different mechanisms, including the inhibition of glucose absorption in the gut or its uptake by peripheral tissues [20]. The antioxidative activities of LE-B, LE-C, and flavonoid compounds (ericiotrin, diosmin, hesperidin, and narirutin) present in citrus fruit peels especially lemon peels may act together with the phytochemicals present in green tea for enhanced

antioxidant levels [21]. The use of citrus peels in green tea can add flavor as some glycosylated flavanones can be easily converted into the corresponding dihydrochalcones, which are potent natural sweeteners [22]. Citrus peel in green tea can be used in lowering the plasma liver cholesterol, serum triglyceride level, serum total cholesterol, liver total lipids, and liver cholesterol. The banana peels have various antioxidative phytochemicals, namely flavanone glycoside-naringin and flavonol glycoside-rutin, dopamine, arginine and catecholamines which might increase antioxidant levels of green tea [23]. Fruit peels have the ability to decrease serum LDL-Cholesterol level, improve glucose tolerance and the insulin response, reduce hyperlipidemia and hypertension, and contribute to gastrointestinal health and the prevention of certain cancers such as colon cancer [24].

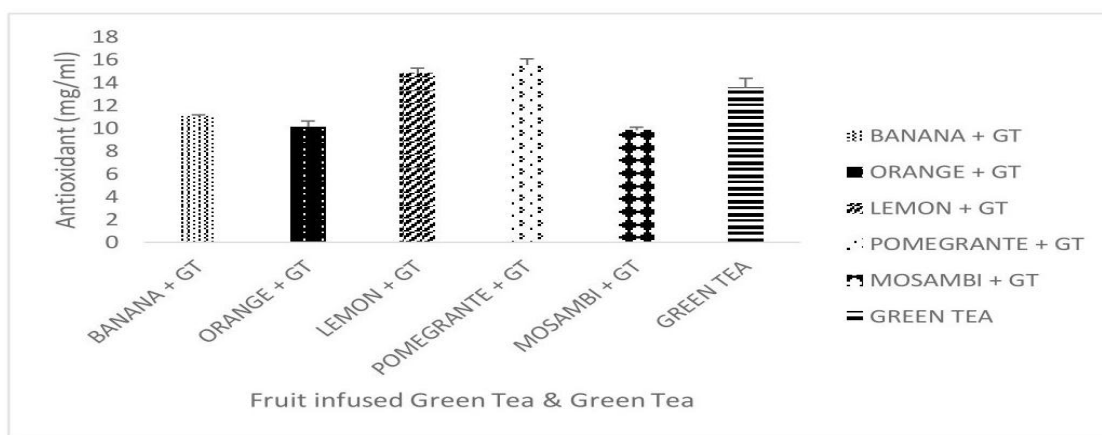


Fig. 2: The bar chart depicts the antioxidant content in fruit peels infused green tea (banana, orange, lemon, pomegranate and mosambi) and green tea in mg AAE/g

4. CONCLUSION

The phytochemical screening of aqueous extract of Kangra green tea with different fruit peels revealed the presence of carbohydrates, proteins and tannins that might contribute to the nutritional value of peels infused Kangra green tea. The presence of the flavanoids and phenolics in the fruit peels infused Kangra green tea suggests the antioxidant activity of this beverage due to their ability to reduce free radical formation and scavenge free radicals. The addition of dried fruits peels to green tea not only enhances the taste and aroma of the brew but may also contribute to its antioxidant properties. Among all tested blends of green tea and dried fruits peels, the highest antioxidant property was found with green tea infused pomegranate peels. Thus, results of the present study confirm the use of fruit peels in Kangra green tea as 'functional ingredient' which might provides numerous health benefits.

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