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POLYPHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, ENZYME INHIBITORY EFFECT AND SENSORY EVALUATION OF NOVEL COMPOSITE TEA

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Abstract
Green tea is consumed all over the world and is well known for its health benefits. The nutritional profile of green tea can further be improved by adding other ingredients to it. Therefore, the objective of the present study was to evaluate the proximate and mineral composition, total phenolic content and antioxidant activity, inhibitory activity on α-amylase and α-glucosidase and sensory evaluation of a novel composite tea made from six different ingredients. The green tea was used as a control. Both the control and novel composite tea samples were first dried and then subjected to further chemical analysis. The proximate composition was assessed by following the procedure of Association of Official Analytical chemists. Mineral composition was analysed by using atomic absorption spectrophotometer. Total phenolic content was measured by using Folin-Ciocalteu method. Total flavonoid was determined by aluminium chloride colorimetric method. Antioxidant activity was examined by ABTS and DPPH method. The activity of tea samples on inhibition of α-amylase and α-glucosidase was measured by enzymatic method. Sensory score was evaluated by using 9-points hedonic scale. The results from the present study indicated that proximate composition including ash, fibre and protein content of novel composite tea were significantly ($p < 0.05$) higher than control tea. The concentration of macro and micro-minerals including Ca, Mg, K, Na, Fe, Zn and Cu of novel composite tea were also significantly ($p < 0.05$) higher compared to control tea. Total phenolic content, flavonoid content and antioxidant activity of novel composite tea were significantly ($p < 0.05$) higher compared to control tea. The results showed higher inhibitory action of novel composite tea against α-amylase and α-glucosidase enzymes when compared with control tea. There was no significant difference in sensory score in term of colour, aroma, taste and overall acceptability between control and novel composite tea. It is concluded from the present study that novel composite tea had maximum bioactive compounds and significantly inhibited the activities of α-amylase and α-glucosidase enzymes.
Keywords: The nutritional profile of green tea, inhibitory action of novel composite tea, micro nutrients, CVD, Hypertension

1. INTRODUCTION

Green tea is one of the most consumable beverages in the world and has been shown to have various health benefits (Zaveri, 2006; Chacko et al., 2010). Recent research studies have proved that green tea extract contain polyphenols such as catchin, epicatchin, epigallocatechin gallate and epigallocatchin which have beneficial effects on health and are responsible for its antioxidant activity (Zhang et al., 2018; Zaveri 2006). Green tea has also shown to promote weight loss by improving fat oxidation and energy expenditure in various research studies (Hursel et al., 2009). However, the interest for herbal tea is also increasing as it possesses significant health benefits (Moodley et

al., 2015). Herbal tea is composed of different traditional herbs. These herbs have been shown to contain antioxidant phenolic compound and are use as flavouring agent to increase the taste and aroma of beverages (Durak et al., 2014).

Cinnamon the most known, traditional and aromatic herb belongs to lauraceae family and is obtained from inner bark of the cinnamomum plant which is use for many heath purposes (Adisakwattana et al., 2011; Ranjber et al., 2006). Cinnamon is known as phenol-rich herb, contains 100mg of total phenolic content per gram and also composed of bioactive ingredient mainly coumarin and cinnamic acid (Klejdus and Kvacik., 2015; Durak et al., 2014). Cinnamon addition to beverages improves the sensory quality of end product due to its aroma while Catechine, procynidine (type A, B) present in cinnamon bark show effective antioxidant potential (Rao et al., 2014). Evidence from research show that cinnamon are beneficial in improving hyperglycemia (Singletary et al., 2008). Cinnamaldehyde is the major and most significant component of cinnamon specie. Cinnamon barks extract inhibit the enzyme activity involved in digestion and this effect is due to the existence of glycoside group, flavonoid and other major metabolites which possess enzyme inhibition potential (Shihabudeen et al., 2011).

Cardamom is polymorphic herb from zingiberacea family of ginger has beneficial effect on health (Das et al., 2012; Goyal et al., 2015; Bajaj et al., 1993). Flavonoids, trepene, ester and oleoresin is the oil content of cardamom whereas oleoresin is the most important having potent antioxidant activity and enormous polyphenol (Aghasi et al., 2018; Kapoor et al; 2008). Cardamom has strong free radical scavenging ability, effective enzyme inhibitory activity and is famous for its pleasant odor, sweet flavour thus improve food palatability (Aghasi et al., 2018; Goyal et al., 2015). Presence of flavonoid, polyphenol and vital lipid mainly sterols attribute towards strong antioxidant property of cardamom which in turn increase enzymatic antioxidant activities of catalase, glutathione peroxidase and superoxide dismutase (Bhatti et al., 2010 Azimi et al., 2014).

Fennel a biennial herb belongs to family Apiaceae has two types piperitum and valgure cultivated mostly in Mediterranean region is a medicinal herb containing mineral, vitamins and mono unsaturated fatty acids (Ahmad at al., 2018; Ahmed at el., 2019). Fennel has high phytochemical and oil content among which anethole and fanchose is main component of oil and is rich in volatile component (Anwar et al., 2009; Ahmed at el., 2019). Polyphenol constituent of the fennel are rosemarinic acid, kaempferol-3-0-glucoside, quercitin-3-0 galactoside (Houhou et al., 2018). Presence of ingredients including limonene, nerole, monoterpene contribute towards fennel seed flavour and hence use as a flavouring agent traditionally (Cautela et al., 2018).

Damask rose is a perennial shrub belongs to Rosaceae family, has therapeutic and ornamental uses and is famous for its amusing aroma (Ulusoy et al., 2009; Boskabady et al., 2011). The presence of abundant polyphenols, flavonoids and natural antioxidant mainly, carotenes, tocopherol and vitamin C make damask rose as an effective antioxidant against free radicals (Ulusoy et al., 2009; Patil at al., 2015). Damask rose has been shown to prevent obesity and high blood glucose level by having strong capacity for inhibition of α-glucosidase enzyme (Gholamhosanien et al., 2009).

Carom seed commonly known as Ajwain is a spice from Apeaceae family and is used as a medicinal herb (Zarshenas et al., 2014). Its antioxidant content has been shown in various studies to inhibit oxidation (Chatterjee et al., 2012). Carom seed is traditionally use for it flavour and fragrance and hence improve sensory quality of food (Nickavar et al., 2014). Carom seed oil contains Polyphenols, antioxidants, flavonoids and terpenes including γ-terpinene, thymol, and chymene (Malekinejad et al., 2012).

Although all herb and spices contain polyphenols and antioxidant compound and have beneficial effects on health. However, these herbs have been added to different beverages and foods in isolation and are not yet used in combined form. Therefore, the aim of the present study was to determine the antioxidant potential and enzyme inhibition effect of a novel composite tea made from the above mentioned herbs. This study determines the approximate and mineral composition, polyphenolic content, and antioxidant activity of a novel composite tea, starch digestive enzymes inhibitory effect of a novel composite tea and evaluate the sensory quality of novel composite tea.

2. LITERATURE REVIEW

2.1 Cinnamon

2.1.1 Cinnamon polyphenols content

Cinnamon is a popular herbal remedy that contains polyphenols, which are responsible for its antioxidant activity. It contains essential oils such as e-nerolidol, alpha terpineol, alpha cubebene, L-bornyl acetate, cinnamon acetate, alpha thujene, ugenol.

2.1.2 Cinnamon antioxidant activity

A study was conducted to determine the polyphenols content in cinnamon fortified yogurt. The results showed that the yogurt had higher polyphenols and significant antioxidant activity. Cinnamon showed significant antioxidant activity and strong inhibitory potential against starch digestive enzymes.

2.1.3 Cinnamon enzyme inhibitory activity

Cinnamon bark extract has effective inhibitory effect on yeast -glucosidase enzyme and -glucosidase activity of mammalians compared to other plant herbs, and this inhibitory function affects digestive and intestinal enzymes and

thus have positive effect on glycemic level. Research was performed to check the enzyme inhibitory effect of four different commercial types of cinnamon. Cinnamon cassia showed highest inhibition and reduced starch digestion during oral and gastric phase of digestion. Cinnamon samples were purchased from local market and extracted using methanol water and ether. The result shows that cinnamon extract possesses strong antioxidant activity and has high content of phenolic compound.
Cinnamon improves glucose metabolism during digestion and helps in insulin resistance. It also improves glucose transportation in muscles and adipose tissue via the glucose transporter process.

2.2 Cardamom

Cardamom is used in eastern, Arab and western countries as aromatic spice and has many medicinal and traditional properties such as anti-inflammatory, gastro protective, anticancer and anti-scavenging.

2.2.1 Cardamom phenolic content

Cardamom oil contains terpenes, esters flavonoids, 1-8 cineoles which decrease lipid profile. Cardamom polyphenols are quercetin, kaempferol, luteolin and pelargonidin which have antioxidant properties. It contains α- Terpinol, citronellol, Camphor, sabinene, linalool, cymene in trace amount

2.2.2 Cardamom antioxidant activity

Cardamom has strong antioxidant potential, with 50-100g of phenolics and flavonoids, and bioactive ingredients including eucalyptol, alpha-pinene, beta-pinene and geraniol having anti-proliferative and anti-invasive properties. A study was performed on four different varieties of cardamom to check the antioxidant activity. The results showed that Malabar variety has significant flavonoid content compare to other varieties, and ethyl extract of Malabar cardamom possesses efficient antioxidant potential.
The cardamom seed and pod extracts show strong antioxidant effect against DPPH. Cardamom ethanolic and aqueous extracts have significant phenolic content and strong antioxidant potential.

2.2.3 Cardamam proximate analysis

Cardamom has high vitamin B, C, iron and protein while low fat content. It also has high phenolic antioxidant contents and linalool, alpha terpinyl and 1,8-cineole are the major components of cardamom.

2.2.4 Cardamom inhibitory activity

Cardamom methanolic extract shows significant antioxidant activity and inhibits starch digestive enzymes, thus helping to control hyperglycemia.

2.3 Fennel

2.3.1 Fennel proximate analysis

Fennel (Foeniculum vulgare) is a medicinal, aromatic and flavoring herb which is beneficial in treating gastrointestinal and respiratory problems. It contains maximum amount of protein, sterols, vitamins, phenols and fibre.

2.3.2 Fennel polyphenols content

Fennel contains phenols, polyphenolic acids, flavonoids and is used to treat cardiovascular diseases, inflammatory disorders and bacterial and fungal infections. A study was conducted to check the effect of incorporation of fennel on the quality of protein bread. The results showed that fennel seed bread had higher moisture content and more hardness than bread without fennel seed.

2.3.3 Fennel antioxidant activity

Fennel seeds contain anti-inflammatory agents, including cis-miyabenol C, trans- miyabenol C, sinapyl glucoside and 4-O- glucoside. Various studies have shown that fennel possess strong antioxidant potential. The polyphenols and antioxidant activity of Chinese and Egyptian fennel seed extract was determined and it was concluded that Egyptian fennel seed extract possessed higher total phenolic content than Chinese fennel seed extract.

2.3.4 Fennel inhibitory effect

The flavonoid polyphenol in fennel has the potential to inhibit starch digestive enzyme and has a direct or indirect role in the control of hyperglycemia. Fennel seed extracts have inhibitory effect on lipid peroxidation and starch digestive enzymes. Ethyl acetate and benzene extract of fennel seed possess strong inhibitory effect against starch digestive enzymes than n-butanol. Fennel extract improves hyperglycemia in streptozotocin-diabetic rats and has strong antifungal activity. It is widely use as anti-diabetic drug.

2.4 Damasc Rose petal

Damask rose is an ornamental plant, used in food and perfume industries, and has therapeutic uses for digestive problems, chest and abdominal pain and inflammation.

2.4.1 Damasc rose chemical composition

Damascene is composed of citronella, phenyl ethyl alcohol, tricosane, eicosane, eugenol, heneicosane, -guaiene, and geraniol. It has anti-bacterial, antimicrobial, and antiviral properties, and is beneficial in preventing abdominal pain, chest pain, and constipation.

2.4.2 Damasc rose inhibitory effect

Damask rose has -glucosidase inhibitory action which suppresses postprandial glycemia in both animal and human studies. Methanol extract of damask rose decreases blood glucose level due to its inhibitory effect against starch digestive enzymes.

2.4.3 Damasc rose mineral analysis

Damask roses contain vitamins, minerals, flavonoids, tannins, carotenoids, phenolic compounds, amino acids, volatile oil, saturated and unsaturated fats. Minerals in damask roses include potassium, magnesium, calcium, phosphorous, zinc, boron, iron, copper, and manganese.

2.4.4 Damasc rose polyphenols

A study was carried out to investigate the antioxidant activity of dry rose tea. The results indicated that the tea had significant anti-oxidant activity, had good sensory quality and was acceptable by the consumer.

2.5 Carom seed

Carom seed is used in the treatment of many diseases like colonic pain and gastroinstestinal problems. Carom seed improves immunity, digestion, respiration and helps in the prevention of gout and rheumatis, it also possesses antibiotic property and boost immune system, and is use as remedy for the digestive, and respiratory diseases.

2.6 Green Tea

2.6.1 Green tea polyphenolic content

Green tea is most commonly consumed product in china, japan and northern areas and provides protection from cancer and cardiovascular diseases.

2.6.2 Green tea proximate analysis

It contains protein 15-20%, amino acids 1-4%, carbohydrate 5-7 in dry weight, calcium, magnesium, iron zinc, chromium, phosphorous, selenium, cobalt, vitamins B, C, E, sterols, caffeine, polyphenols.

2.6.3 Green tea antioxidant property

Green tea has highest polyphenolic content (5 GAEg/ml) and possess nutritional effect. It also has significant antioxidant property than vitamin C, E, carotene and tocopherol.

2.6.4 Green tea enzyme inhibtion

A research was conducted to determine the inhibition of -amylase by green tea catchines and amino acids. The inhibition ratio was 61% when green tea contained catchines, epicatchines, epigallocation, epigallocatchine gallate, epicatchine gallate.

3. MATERIALS AND METHODS

The research was conducted in the Human Nutrition department at Agriculture University Peshawar. Novel composite tea was prepared from five ingredients including cardamom, Iranian rose petal, cinnamon, carom seed and fennel seed. These ingredients were mixed in a ratio of 1.5: 5: 6: 10: 10 respectively. Green tea and the mixture of these ingredients were mixed at 50:50. For sample extraction 10 g tea sample was added to 100 ml distal water and then kept in water bath at 95oC for 30 minutes. After this the sample was centrifuged for 20 minutes at 3000 rpm. The sample extracts were then kept in the freezer until use.

3.3 Chemical Analysis

3.3.1 Proximate Analysis

Moisture, ash crude protein and crude fiber of both tea samples were assessed by following the procedure of AOAC (2000).

3.3.1.1 Moisture determination:

Moisture content was determined by using oven drying technique. First the clean, dry and labeled petri dishes were weighted and then 5g sample was added to these pre-weighted petri dishes and kept in oven for 4 hours at 105°c for drying. After removing from oven the petri dishes were kept in desiccator for cooling. After cooling the final weight of petri dishes were noted. The formula used for moisture content determination was:

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_1} \times 100$$

Where

W_1 = Fresh sample weight

W_2 = Sample weight + Petri dish weight before drying

W_3 = Sample weight + Petri dish weight after drying

3.3.1.2 Ash determination

Muffle Furnace (NeyVulcan™) was used for the determination of ash content in both samples. First the weight of empty dry crucible was noted and then 2g sample was transferred to the cleaned and dried crucible. The sample was burnt in open fire at 550°c and then the crucible was kept in furnace at 550°c for 4 hours. When the temperature falls to 100°c the furnace was opened. The crucible was removed when the sample colour change into grayish white ash.

After the sample is converted into ash the crucible was kept into desiccator for cooling. The final weight was recorded and formula used for ash determination was

$$\% \text{ Ash Content} = \frac{W_3 - W_1}{W_2} \times 100$$

Where
W_1 = Empty crucible weight
W_2 = Sample weight
W_3 = Crucible weight + sample weight after ashing

3.3.1.3 Protein determination:

Kjeldhal apparatus was used for the analysis of protein content. For protein content determination 0.5 gram sample was weighted and transferred to digestion tube. For digestion 2-3 gram catalyst (potassium sulfate 93g + copper sulfate 7g) was added to the digestion tubes and were shake in order to mixed the sample and digestion mixture. Then 10ml concentrated H2SO4 was added to the digestion tubes and digestion tubes was kept in digestion assembly until the colour change to light green. Then the digestion tube cooled and distilled water was added in order to attain the 100ml volume and was stored in plastic labeled bottles. The distillation of digested sample (5ml) was performed by using 40 % NAOH (5ml) and the distillate was added to 2 % boric acid(10ml) and 2-3 drops of methyl red as indicator was added to it. Titration was done with H2SO4 solution. The formula used for protein content determination was

$$\text{Nitrogen} = \frac{(TR - Br) \times N \times \text{Nitrogen atomic weight} \times DF}{\text{Weight of sample} \times V} \times 100$$

$$\% \text{ Crude Protein} = \% \text{ Nitrogen} \times \text{conversion factor of nitrogen to protein}$$

TR = Sample titration reading
Br = Blank reading
N = H2SO4 normality (0.02)
V = Volume taken for distillation
DF = Dilution factor
Nitrogen atomic weight = 0.014

3.3.1.4 Fiber determination

Dilute acid and alkali hydrolysis method was used to assessed the crude fiber content of samples. 2g dried sample was transferred to beaker and to it 100ml H2SO4 was poured and was heated and condenser was placed on beaker. When it start boiling the time was recorded and NAOH was added after 30 min. the solution was then filtered by crucible attached with filtration apparatus and acetone was used for rinsing. The residue was then kept in an oven for overnight at 100°c and after drying weight was recorded. The dried residue was then kept in furnace for 2 hours at 550°c and after ashing weight was recorded. The formula used for fiber determination was

$$\% \text{ CF} = \frac{W_2 - W_3}{W_1} \times 100$$

Where
W_1 = Sample weight
W_2 = Sample weight after drying
W_3 = Sample weight after ashing

3.3.2 Mineral Analysis

Mineral content (Zn, Fe, Cu, Ca, Mg, K, Na) of novel composite tea and control tea were determined by using inductively Coupled Plasma Atomic Emission Spectrometry.

3.3.2.1 Sample Preparation for mineral analysis:

for mineral analysis first sample extraction was done by following the AOAC (2000) procedure. 1 g sample was taken in a conical flask and to it 10 ml of nitric acid and per chloric acid was poured and the flask was then kept in digestion chamber until the appearance of white fumes and when the solution became clear. The clear digested solution was then cooled and to attain the final volume of 100ml distilled water was added to the solution and was stored in pre labeled plastic bottles.

3.3.2.2 Mineral Analysis:

Mineral analysis of novel composite tea was performed by using Atomic Absorption Spectrophotometer with specific lamp and flow burner. The digested samples were placed in spectrophotometer with the help of cuvettes.

3.3.3 Sample Extraction for Total Phenolic, Total Flavonoids and Antioxidant Activity

Method of Dhanani et al (2017) was used for sample extraction. Sample was weighted by using air tight balance and added to pre-labeled plastic ependorf centrifugal tubes. In ependorf tubes 1% HCL in methanol (10ml) was added. These tubes were then placed in water bath shaker and were shaked in dark for 2 hrs in order to protect the sample from light. Then the sample was centrifuge for 20 min at 3500rpm in centrifuge machine and after centrifugation the supernatant was taken with micro pipette and was added to tubes with aluminum foil and stored in freezer at -20 for further analysis.

3.3.4 Determination of Total Phenolics

The reagents used for the determination of total phenolics included

Folin-Ciocalteu reagent (10 fold), sodium carbonate (Na_2CO_3 7.5%), gallic acid (0-5mg/100ml) and methanol .

Follin-ciocalteu (FC) reagent method of Singleton et el., (1999) was used for total phenol determination. 20ml FC reagent and 180ml of distilled water was added to prepare FC (10 fold) solution. 15g sodium carbonate and 200ml distilled water was added to made 7.5% of sodium carbonate. With the help of pipette 2ml of sample extract was taken in flask for determination of total phenolics. 10ml of FC (10 folds) reagent was added to the flask using graduated cylinder. After 3min, 8 ml Na_2CO_3 (7.5%) was added and final volume was made to 25ml by adding 5 ml distilled water. The final mixture was stored for 1 hour at room temperature in dark. Through Spectrophotometer at 765nm wavelength in triplicate absorbance of final solution was determined. Using distilled water at same wavelength initially absorbance of blank was measured. Gallic acid was used as a standard and the results were expressed as mg GAE/ 100g dry weight.

3.3.5 Determination of Antioxidant activity

Antioxidant activity was assessed by two method including ABTS (2,2-azino-Bis-3-Ethylbenzothiazoline-6-sulfonic Acid) and DPPH (2, 2-diphenyl-1-picrylhydrazyl).

3.3.5.1 Antioxidant analysis by ABTS

ABTS and methanol reagent were used. With micro pipette 150 µl sample extract was taken for determination of antioxidant activity. Then 2.85 ml of ABTS was added to sample extract and kept in dark at room temperature for 30 min. Absorbance was recorded at 734nm wavelength through spectrophotometer. Absorbance of control was also determined in which sample extract was replaced with methanol (150µl). The results were stated as percent inhibition (% I) of antioxidant activity. For determination of antioxidant activity the following formula was used:

$\% I = [(Ac – As)/Ac] \times 100$

Where A_C = Absorbance of control

A_S = Absorbance of sample

3.3.5.2 Antioxidant analysis by DPPH

Methanol and DPPH (0.1M) reagent were used. The method of Yang et al., (2012) was followed for antioxidant activity determination. 10mg DPPH was added in 250ml methanol to prepare DPPH (0.1M) stock solution. The stock solution was covered with almunium foil and stored in dark. From stock solution 2.85ml DPPH was taken in conical flask covered with aluminium foil and 150µl of sample was added to it and stored in dark for 30 min and then at 517 nm wavelength absorbance was measured . The results were stated as percent inhibition (% I) of antioxidant activity For determination of antioxidant activity the the following formula was used:

$\% I = [(Ac – As)/Ac] \times 100$

Where A_C = Absorbance of control

A_S = Absorbance of sample

3.3.6 α- amylase inhibition

The method of Miao et al. (2015) with slight modification was used to determine α- amylase inhibition. Starch solution was prepared by adding 0.5 gram starch in 50 ml buffer and then kept in oven for 30 min at 100 °C. DNS solution was prepared by dissolving 1g of DNS in 2 M NAOH and 30 g of potassium sodium tartrate in 50 ml distal water. Both solution were then added, mix and heated. Volume of solution was then increased to 100 ml by adding distal water. For 10 g sample 0.02 M sodium phosphate was mixed in 500 µl sample extract to prepare the mixture. 50 µl α-amylase was added to mixture and was incubated for 10 min at 25°C then 500 µl starch solution was added. Again incubation was performed for 25°C for 10 min. After incubation DNS (1 ml) were added and sample was incubated in oven at 100 ºC for 5-10 min. After sample was cooled to room temperature, 10 ml distilled water was added and absorbance was measured at 540 nm. Same procedure was followed for control sample but 500 µl buffer solution was taken instead of sample extract. For control blank 500 µl buffer solution instead of sample extract was used and 50 µl α-amylase was not added in the solution. For sample blank starch solution, sample and DNS were added.

The following formula was used to calculate percent inhibition (%I).

$\% I = (Ac – A_{CB}) - (A_S – A_{SC})/ (A_C – A_{CB}) \times 100$

Where C is for control, CB for control blank, S for sample and SC for sample control

3.3.7 α-glucosidase inhibition method

The method of Godavari et al (2018) with slight modification was used to determine the α-glucosidase inhibition. Sucrose solution was prepared by adding 2 g of sucrose in 100ml buffer. 50 µl sucrose solution, 50 µl sample extract and 50 µl α-glucosidase was added to make the solution. The solution was incubated at 37 ºC for 60 min and kept in oven for 10 min. GOPOD (3 ml) were added to the solution and incubation was done for 5 min at 37 ºC Absorbance was measured at 505 nm. For control sample 20 µl buffer was added instead of sample. For control blank replace sample, sucrose and enzyme with buffer. For sample blank replace sucrose and enzyme by buffer

The following formula was used to calculate percent inhibition (%I).

$\% I = (A_{CB} – A_C) – (A_{SB} – A_S) / (A_{CB} - A_C) \times 100$

Where CB is for control blank, C for control, SB for sample blank and S for sample.

3.8 Sensory evaluation

Sensory evaluation was carried out in Human Nutrition Department at Agriculture University Peshawar. 50 untrained students (males and females) were selected and for sensory evaluation 9-point hedonic scale was used. The subjects were requested to fast for 3 hours before evaluation session. All samples were evaluated for taste, appearance, colour and overall acceptability. A sample was considered acceptable if the mean overall acceptability score was equal or greater than 6.

3.9 Statistical analysis

All statistical analysis was carried out using SPSS software version 21. Independent sample t-test was used to determine the difference in proximate and mineral composition, polyphenol content, antioxidant activity, enzyme inhibition activity between control and novel composite tea. Paired sample t-test was used to determine the difference in sensory parameters between green tea and novel composite tea. All analysis was carried out at $p \leq 0.05$

4. RESULTS AND DISCUSSION

In the present study the proximate and mineral composition, total phenolic content, total flavonoid content, antioxidant activity and inhibitory effect on α- amylase and α- glucosidase activities of a novel composite tea was examined. The novel composite tea was prepared from five ingredients that included cardamom, Iranian rose petal, cinnamon, carom seed and fennel seed. These ingredients were mixed in a ratio of 1.5: 5: 6: 10: 10 respectively. Then green tea and mixture of these ingredients were mixed at 50:50 ratios and were then subjected to further analysis.

4.1 Proximate composition of tea:

he proximate composition of novel composite tea was higher than green tea in terms of ash, protein and fiber content, and no significant change in moisture content was observed between the two tea types. The higher content of these nutrients in the individual ingredients may explain the higher fiber, protein and ash content. Fennel seed and carom seed have higher protein, fiber and ash content compared to control bread.

Table 4.1. Proximate composition of green and novel composite tea samples (% dry basis) *

Composition	Green tea	Novel composite tea
Moisture	6.41 ± 0.92^{a}	6.79 ± 8.46^{a}
Ash	3.45 ± 0.43^{b}	5.12 ± 0.56^{a}
Protein	2.76 ± 0.60^{b}	5.79 ± 0.57^{a}
Fiber	5.42 ± 0.85^{b}	9.84 ± 0.70^{a}

*Values are means ± SD of duplicate analyses.
Means in the same rows with different letters are significantly different. $P < 0.05$ (Independent sample t- test).

4.2 Mineral composition of tea samples

Table 4.2 shows the mineral composition of green and novel composite tea. The results show that novel composite tea had higher magnesium, potassium, calcium and sodium content, while iron content was highest among micro minerals followed by zinc and copper. Carom seed contains potassium, copper, zinc, iron and manganese in sufficient amount. Fresh damask rose petal has higher mineral content than black tea.

Table 4.2. Mineral composition of green and novel composite tea samples (mg/100g dry basis) *

Mineral	Green tea	Novel composite tea
Ca	0.84 ± 0.23^{b}	1.95 ± 0.36^{a}
Mg	1.03 ± 0.40^{b}	2.99 ± 0.37^{a}
K	1.66 ± 0.35^{b}	2.84 ± 0.40^{a}
Na	0.46 ± 0.38^{b}	1.64 ± 0.32^{a}
Fe	1.05 ± 0.11^{b}	1.70 ± 0.13^{a}
Zn	0.13 ± 0.19^{b}	0.39 ± 0.15^{a}
Cu	0.04 ± 0.02^{b}	0.08 ± 0.03^{a}

*Values are means ± SD of duplicate analyses.
Means in the same rows with different letters are significantly different. $P < 0.05$ (Independent sample t- test).

4.3 Total phenolic and total flavonoid contents of green tea and novel composite tea:

Table 4.3 represents the total phenolic and total flavonoid content of green tea and novel composite tea. Novel composite tea had significantly higher TPC and TFC compared to green tea. Cinnamon contains total flavonoid and phenolic content in higher concentration in its root, stem, leaf and seed. Previous studies have shown that cardamom contains high amount of total phenolic and flavonoid content, and fennel and carom seeds also contain high amount of phenolic compound and possess strong antioxidant activity. The total phenolic and flavonoid content of wild fennel and cultivated fennel were higher in cultivated fennel.

Table 4.3. Total phenolic and total flavonoid contents of green and novel composite tea samples (dry basis)*

Sample	Total phenolic content (mg GAE/100g)	Total flavonoid content (mg QE/100g)
Green tea	296.45 ± 5.92^{b}	134.03 ± 5.14^{b}
Novel composite tea	430.42 ± 6.32^{a}	214.82 ± 6.21^{a}

*Values are means ± SD of triplicate analyses.
Means in the same columns with different letters are significantly different. $P < 0.05$. (Independent sample t- test).

4.4 Antioxidant activity of green and novel composite tea samples:

The antioxidant activity of novel composite tea was significantly higher compared to green tea. This was due to the higher total phenolic content. A study was conducted to determine the antioxidant activity of cinnamon, cardamom and ginger tea. Cinnamon showed the highest antioxidant activity followed by cardamom tea and ginger tea.
In another study, cardamom tea significantly reduced oxidative stress in blood and improved antioxidant status. It also showed higher antioxidant activity when measured in different concentration of methanolic extracts. Fennel was assessed for its antioxidant activity in a previous study and was concluded to exhibit efficient antioxidant activity and play a role in the inhibition of peroxidation.

Table 4.4. Antioxidant activity of green and novel composite tea samples (dry basis)*

Sample	ABTS (µmol TE/100g)	DPPH (µmol TE/100g)
Green tea	306.53 ± 6.02^{b}	251.23 ± 6.64^{b}
Novel composite tea	515.67 ± 7.13^{a}	304.82 ± 6.81^{a}

*Values are means ± SD of triplicate analyses.
Means in the same columns with different letters are significantly different. $P < 0.05$ (Independent sample t- test).

4.5 Enzymes inhibitory effects of green and novel composite tea samples

The inhibitory effect of novel composite tea on -amylase and -glucosidase was significantly higher compared to green tea. The result of the present study is supported by a previous study in which cinnamon inhibited the digestion of starch and may have a beneficial effect on hyperglycaemia.
Another study was conducted to evaluate the inhibitory effect of Chinese cardamom and Indo Pak black cardamom on -amylase and -glucosidase and slowed the digestion of starch. Another study proved that carom seed oil and ethanolic extract of carom have antidiabetci effect because it inhibits -glucosidase enzyme. This helps in reducing blood glucose level. Damsc rose extract showed inhibitory activity against -glucosidase, which may have slowed down the digestive process and decreased blood glucose.

Table 4.5. α- amylase and α-glucosidase inhibitory activities of green and novel composite tea samples

Sample	α- amylase (% inhibition)	α-glucosidase (%inhibition)
Green tea	43.13 ± 2.82^{b}	31.61 ± 4.24^{b}
Novel composite tea	65.27 ± 3.02^{a}	54.22 ± 4.86^{a}

*Values are means ± SD of duplicate analyses.
Means in the same columns with different letters are significantly different. $P < 0.05$ (Independent sample t- test).

4.6 Sensory evaluation tea samples

The sensory evaluation of control tea and novel composite tea showed no significant differences for any of the sensory parameters when control and novel composite tea were compared. Previous research studies have shown that cinnamon and cardamom are safe to consume and are used as flavouring agents in different food products. A study conducted on fennel seed showed that it is an aromatic herb and is used widely as a flavouring agent in different food products. Its incorporation in bread increases its acceptability.

Table 4.6. Sensory evaluation of green and novel composite tea samples (n = 50)*

Sample	Color	Aroma	Taste	Overall acceptability
Green tea	7.52 ± 1.26	7.92 ± 1.62	7.84 ± 1.58	7.86 ± 1.33
Novel composite tea	7.34± 1.37	7.46 ± 1.52[b]	7.56 ± 1.92	7.68 ± 1.67

Values are means ± SD.
Values for any of the sensory parameters are not significantly different. $P < 0.05$ (paired t-test).
*Data collected on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely).

5 CONCLUSION

Based on the findings of the present study, it can be concluded that Novel composite tea had a significantly higher fiber and mineral content than the control tea. Moreover, novel composite tea significantly increased total phenolics, antioxidant activity, and enzyme inhibition activity when compared to control tea. Additionally, the quality of novel composite tea was assessed on a 9-point hedonic scale and no significant difference was found between the novel composite tea and the control tea.
According to the study, the novel composite tea demonstrated significant levels of bioactive compounds as well as efficient inhibition against starch digestion enzymes. As a result, the consumption of this product would be helpful in controlling hyperglycemia. Further studies are now recommended to determine the effect of novel composite tea on biomarkers of cardiovascular health in humans.

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