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Original Article

The Effects of Adding Milk on Antioxidant Activities of Green and Black Tea

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ABSTRACT
The aim of present study, eleven different types of commercial tea samples were assayed to phenolic contents and antioxidant activities. For tea infusion , 2 g of green and black tea were infused in 1000 ml of boiling water in a flask for 30 min, and for preparation of milk with tea , 50 ml of prepared tea was mixed with 50 ml of pre-warmed fresh cow milk with 4.2% fat . After 30 min, the infusions were filtered through a filter paper. Total phenolic and flavonoids content was measured according to the Folin-ciocalteus and aluminum chloride methods respectively. Total antioxidant activity was estimated by different assays such as 2,2-diphenyl-1-picrylhydrazyl radical DPPH ,Trolox equivalent antioxidant capacity (TEAC). Total polyphenols, and antioxidant activity were significantly (P<0.05) different in the commercial tea samples. Green tea had the maximum amount of total phenols, Flavonoids, and antioxidant activity. The addition of cow's milk increased the antioxidant activity of green and black tea preparations. Generally, antioxidant activities of tea+ milk infusion approximately 2 .1 fold greater than black and green tea. Both green and black tea infusions released high level of total phenols and antioxidants into the boiling water within 30 min compare to boiling water in duration of 10 min. For acquires of the highest antioxidant activity and therefore greatest health value, infusion with water at boiling point for up to 30 min ina flask was recommended .
Key words: *Antioxidant activity, DPPH, total phenols, Flavonoids,milk+ tea*

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INTRODUCTION

Tea, *Camellia sinensis*is one of the most beverages which consumed daily routine by most people after water. It grows best in tropical and subtropical areas with different levels of total phenol mostly flavonoid compounds. Phytochemical component of grown tea plant depended to climate to which it adapts. Black tea is a fermented and market economically which drank in Western countries [1].

Green tea is non-fermented type with high antioxidant activity that consumed extensively by China and Japan peoples. Antioxidant potential of tea polyphenol compounds particularly flavonoids widely studied in literatures. The flavonoids have potent antioxidant activity, metal chelating potential and therefore have been suggested for health benefits such as cancer and coronary heart disease risk reduction and also responsible for the characteristic taste and color of tea [2].

Flavones or Flavans belong to flavan-3-ols are most important compounds however;flavonols and flavones were present in lesser amounts in tea flavonoids. The type and concentration of tea flavonoids depends to leaves processing in tea production [3,4].Based on the industrial method ,teas are categorized into two main types; non-fermented and fermented. Innon-fermented (green tea), due to drying and steaming of the fresh leaves polyphenol oxidase was inactive and therefore, oxidation rate in leaves is low and the activity of enzymes stopped and result in, the majority of catechins substances were intact [5].

In fermented ('black tea) type, fermentation stage was occurred before drying and steaming and thus, oxidization of catechin was carried out by active polyphenol oxidase in controlled temperatures and humidity. Result in, polymerisations of catechins were occurred and theaflavins and the arubigins instead of catechin were induced [6].According to some studies phenolic compounds in tea reduce the human cardiovascular diseases and cancer mortality. Antimicrobial activity and antiallergic effect of tea reported in literature [7].In a number of countries such as Iran

and India in some area, tea is usually consumed with milk. According to some in vitro study, milk proteins interact with tea polyphenols and reduce total antioxidant activity. However, nowadays, unclear whether consuming tea with milk significantly changes the biological activities of tea [8].
In Iran, tea is cultivated in the northeast part of the country. Most of the production is commercialized in local
Markets whereas, the remaining was exported.The present study aimed to estimate the phytochemical components and antioxidant properties of different tea brands. Antioxidant activity of tea infusions was determined in presence and absence of milk using two common synthetic free radicals scavenging potential.

MATERIALS AND METHODS

Tea samples were purchased at central market in Ysuj city .A set of eleven commercial different brands tea samples consisting seven fermented (black), commercial Iranian teas , two , non-fermented (green) tea Indian brand and three Indian black tea from different tea factories were analyzed. All biochemical analysis was carried out in triplicate.
The samples (2 g) were infused in 1000 ml of boiling water in a flask for 30 min.for preparation of mixture milk with tea, 50 ml of tea mixed with 50 ml of pre warmed fresh cow milk with 4.2% fat . After extraction 30 min, the infusions were filtered through a filter paper No. 1.
No.3, 4 green Indian tea, No.1and 8 were Indian black teab rand and others were local Iranian black tea brand.
1= Indian (Tajmahal) , 2=KalehAsbi ,3= Indian green tea 4= India green (Lamsa) , 5=Silani, 6= Golden, 7= milad1 , 8= India , 9= kale morcheh ,10= Ahmad , 11 = milad 2
Determination of total flavonoid content**:** Thetotal flavonoid content was determined with aluminum chloride ($AlCl_3$) according to method (Kosalec et al., 2004). The total flavonoid values were determined in terms of Rutin equivalents/g tea[9].
Determination of total phenolic compounds: The total phenolic contents of extracts were determined using Folin-Ciocalteau method with some modifications Total phenol was expressed as Gallic acid equivalent (GAE) / g tea[10].
Antioxidant activity of Dipheny-picrylhydrazyl(DPPH) : The antioxidant activity of extract assessed with little modification. Percent of inhibition was calculated as follow: % Inhibition = $[(A_0 - A_1)/A0] \times 100$ [11].
A_0 is the absorbance of control and A_1 is the absorbance of the plant extracts.IC_{50} or Inhibition concentration in 50% also was calculated .IC_{50} is the maximal concentration of extract to cause 50% inhibition of free radicals activity or damages..
Trolox equivalent antioxidant activity (TEAC): The antioxidant activity was measured using TEAC based on Re method with some modification. Percent of inhibition same DPPH method was calculated. IC_{50} or Inhibition concentration in 50% also was determined [12].
Statistical analysis
Statistics analysis between different groups was accomplished by analysis of variance using the ANOVA and followed by post hoc tests. The data were expressed as mean ± (SD). *P*-values less than 0.05 were considered significant.

RESULTS

Green tea infusion had the highest total phenolic and flavonoids concentration. Total phenol contents of green tea infusion (15.75 g % GAE) and flavonoids (9.5 g% Rutin) was determined. total phenols (10.1-14.25 mg % GAE) and flavonoids (7.3-9.35 mg% Rutin) content Black tea infusions was reported less than green tea Figure 1.The mean of total phenolic contents in tea preparations atboiling after 10 min (8.44 mg % GAE) and boiling temperature after 30 min (12.62 mg % GAE) were estimated. Both green and black tea infusions released high level of total phenols and antioxidants into the boiling water within 30 min compare to boiling water in duration of 10 min. In present experiment a high relationship with correlation coefficients (r^2) =0.7184 was reported between the flavonoid content and antioxidant activities by DPPH assay (Figure 2.The addition of cow's milk increased the antioxidant activity of green and black tea preparations.
The antioxidant activity by TEAC in tea infusions (0.5-1.2) and in tea + milk blend (1.5-2.5 mmole trolox /g tea)was measured. The mean of antioxidant activity in TEAC method in tea preparations (0.95) and in tea + milk combination (2.1 mmole trolox /g) were reported Figure 3.Similarly, the

DPPH free radical scavenging potential of tea extracts (0.83-1.4) and in tea + milk mixture (1.07-2.9 mmole trolox /g) was demonstrated. The mean of antioxidant property ofDPPH assay in tea preparations (0.96) and in tea + milk (2.54 mmole trolox /g) were reported Figure 4.The mean of DPPH radical scavenging of tea preparations at room (29.5%) and boiling temperature (45.1%) were estimated Figure 5.

Green tea infusion and Green tea with milk had the highest antioxidant activity by DPPH and TEAC assays. The antioxidant activity measured by TEAC same DPPH for all tea extracts. There was no significantly different antioxidant activity between DPPH and TEAC methods in every tea extracts. According to results, there was high correlation between two antioxidant methods (R=0.89).

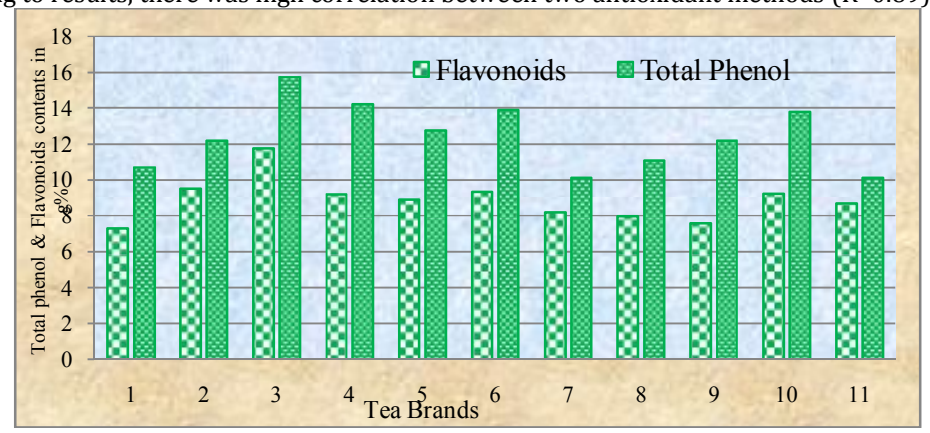

Figure1.Total phenol and flavonoid content of different Iranian and Indian tea brands.
1= India(Tajmahal) , 2=kalehAsbi ,3= India green tea 4= India (Lamsa) , 5=Silani, 6= Golden, 7= milad , 8= India , 9= kale morcheh ,10= Ahmad , 11 = Milad 2

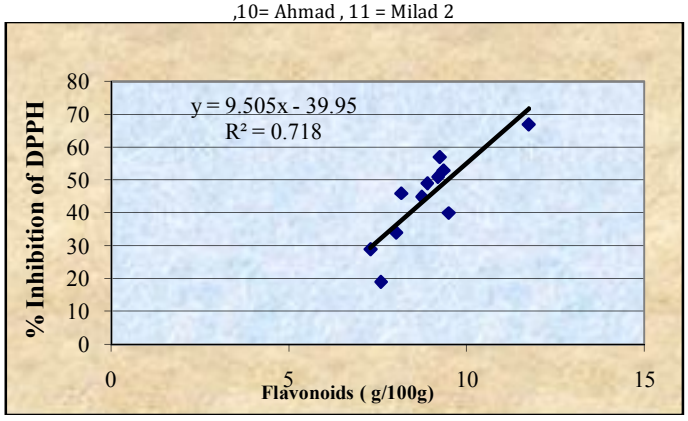

Figure2.Correlation of flavonoids content with antioxidant activity by Diphenyl Pycryl Hydrazyl (DPPH) method in different Iranian and Indian tea brand

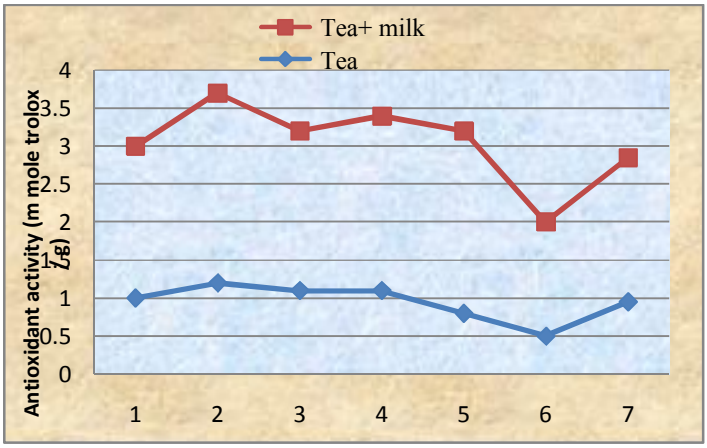

Figure3.Antioxidant activity of different Iranian and Indian tea brands with Trolex equivalent antioxidant capacity (TEAC) in presence and without fresh cow milk (total lipid 4.2%).
1=KalehAsbi , 2 = Indian green tea 3 =Silani,4 = Golden, 5= Milad , 6 = Kale morcheh ,7 = Ahmad ,

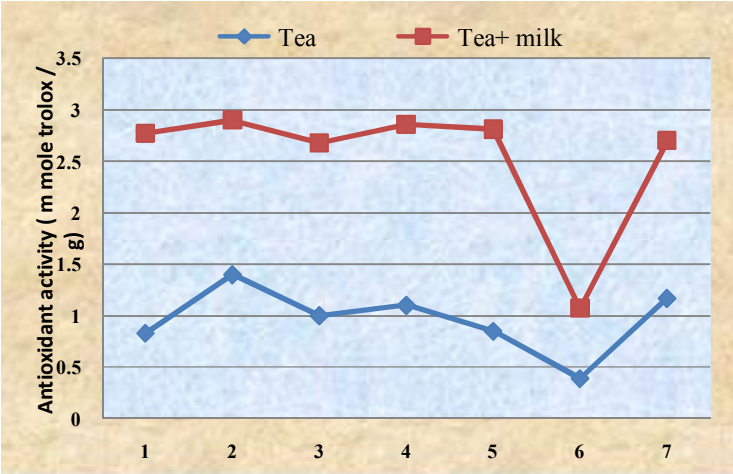

Figure 4.Antioxidant activity of different Iranian and Indian tea brands with DiphenylPycrylHydrazyl (DPPH) in presence and without fresh cow milk (total lipid 4.2%).

1=KalehAsbi , 2 = Indian green tea 3 =Silani,4 = Golden, 5= Milad , 6 = Kale morcheh ,7 = Ahmad ,

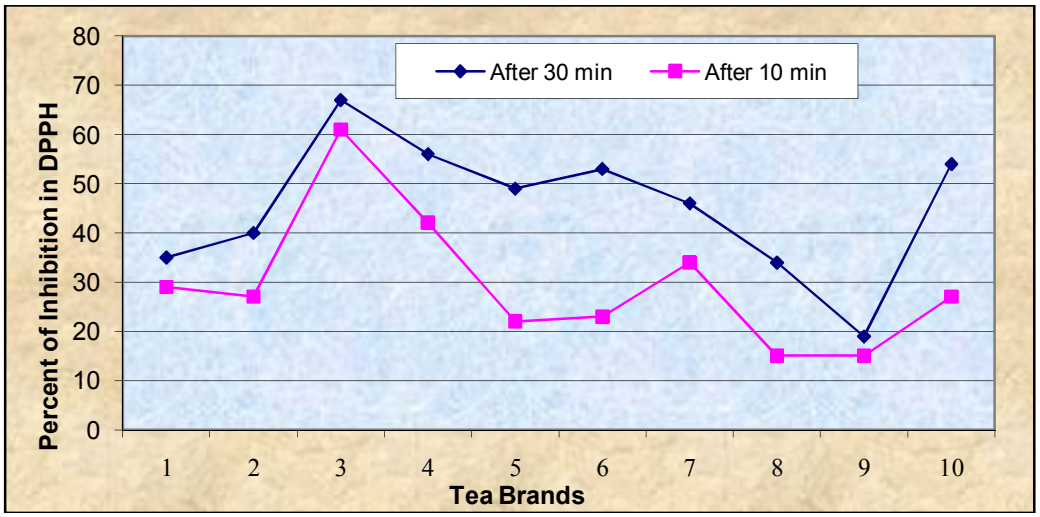

Figure 5.Antioxidant activity of different Iranian and Indian tea brands with DiphenylPycrylHydrazyl (DPPH) in 10 and 30 min.

1= India(Tajmahal) , 2=kalehAsbi ,3= India green tea 4= India (Lamsa) , 5=Silani, 6= Golden, 7= milad , 8= India , 9= kale morcheh ,10= Ahmad .

DISCUSSION

According to present research, Indian tea was comparatively higher total phenol and flavonoids content than those reported for different brands commercialized in Iran. The differences observed between brands may be due to a post maturation process where black tea laststo ferment [13].

Other authors have described the total phenol concentration in Australian black tea, with average of 16%, which relatively higher than the average reported in present study(12.62%), [*14*].In this study, the total phenol content of green tea preparations (3, 4)was reported higher than that found in black tea samples. Usually, Indian teas varieties (*Camellia sinensis*var. assamica) have higher total phenol concentration than Chinese variety (*Camellia sinensis*var. sinensis, [*15, 16*].

The total phenol content of green tea from brand 3in recent study 15.75 % GAE was lower than to that reported for green tea samples from China (21%-23%), one of the principal tea manufacturers of the world (*15*.Antioxidant potential and total phenol concentration are two important tests for quality evaluating regarding its biological potential. These parameters are necessary for the quality control of industrial and imported teas. Referencing to the present results, tea from Yasuj was high quality comparing to tea from other markets. This is the first analysis of teas from the Iranian market in Yasuj area.

According to some studies, interactions between tea phenolic compounds and milk proteins induced decrease in antioxidant activity in vitro [8].

In present study antioxidant activity were significantly increased in presence of milk with tea. The adding of milk to black tea did not largely change plasma catechins[17] or flavonols[18] levels in human volunteers, signifying that addition milk to tea does not significantly affect the bioavailability of tea catechins or flavonols.There are different descriptions for addition milk to tea in animal models. Two human's studies reported to decrease of antioxidant activity after consumption of milk with tea, whereas another research reported no effect. In two studies reported, addition milk to black tea did not decrease its inhibitory potential of carcinogens [19, 20].

The potent antioxidant activities of tea attributed to phenolic compounds particularly to their hydroxyl groups. Hydroxyl groups were required for antiradical activity. These added groups increase donate protons and result in antioxidant activity of compounds. Gallic acid is a potent hydrogen donator to DPPH system therefore, presents high antioxidant activity in black and green teas. This clarifies why antioxidant potential is high in the gallocatechins compounds in green tea [21].

In this research similar to other studies, Green tea had considerably ($p< 0.05$) higher antioxidant capacity compared to black tea [22]. There was no statistically difference in the antioxidant potential of green and black tea by DPPH and TEAC assays.

CONCLUSION

Black and green teas evaluated in present research displayed different level of antioxidant capacity with antiradical activity via DPPHand TEAC methods. However, green tea revealed highest antioxidant activity even in presence milk. Infusion time was reported an important parameter for release of phenolic compounds and antioxidant potential.

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