COMPARISON OF GREEN AND BLACK TEA ANTIOXIDANT ACTIVITY WITH LIPPIA CITRIODORA

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ABSTRACT

Introduction and Aim: Tea, Camellia sinensis family Theaceae, is the second beverage consumed daily in the world for centuries. Lippia citriodora (Verbenaceae) is a medicinal plant that mostly used as a spice with different biological activity which grows in South America and North Africa and Southern Europe. The aim of recent study was managed to compare the antioxidant properties and phenolic contents of 2 commercial green and 4 black tea brands in Iran with Lippia. citriodora as a new beverage. Materials and Method: The samples (2 g) were infused in 1000 ml of boiling water in a flask for 30 Min. After extraction, the infusions were filtered through a filter paper No. 1. Total phenolic and flavonoids content was measured according to the Folin-ciocalteus and aluminum chloride methods respectively. Total antioxidant activity were measured by 2,2-diphenyl-1-picrylhydrazyl radical DPPH method. Statistics analysis between different groups was accomplished by analysis of variance using the ANOVA and followed by post hoc tests. Results: Green tea infusions and Lippia citriodora had the highest antioxidant activity (50.3-54%), total phenol (12-16 g % GAE) and flavonoids (8.5-9.8 mg Rutin %) contents. There was a positive correlation between antioxidant activity and total flavonoid content. Conclusion: Tea infusions and Lippia citriodora leaves extract were demonstrated potent of antioxidant activity and high level phenolic compounds. Lippia citriodora infusion with high flavonoid content and antioxidant activity has been comparable with Iranian commercial brands tea in Yasuj Iran.

Key words: Antioxidant activity, DPPH, total phenols, Flavonoids, Camellia sinensis, Lippia citriodora
INTRODUCTION
Tea, *Camellia sinensis* family *theaceae* is the second beverages consumed daily in the world for centuries. It grows well in tropical and subtropical areas such as South and South East Asia, Africa and the Middle East with different levels of total phenol and flavonoid compounds. *Camellia sinensis* plant produce different forms of tea including, green, black, oolong and white tea. In Black tea production process oxidation is promoted due to substances are oxidized and fermented and market economically which drank in Western countries [1-3].

In fermented (black tea) type, fermentation was formed before steaming process and catechin was oxidized by polyphenol oxidase in controlled condition and theaflavins and the arubigins were formed [4]. In green tea (non-fermented type) oxidation of green leaf polyphenols was not occurred. It has high antioxidant activity that consumed extensively by China and Japan peoples.

In green tea in drying and steaming process, polyphenol oxidase of the fresh leaves was inactive and therefore, oxidation rate in leaves is low and the activity of enzymes stopped, result in, the most of catechins compounds were intact [5]. The type and concentration of tea flavonoids depends to leaves processing in tea production.

In human research on epidemiological studies, antioxidant potential of tea polyphenol particularly flavonoids widely studied. The flavonoids have potent scavenging reactive oxygen and chelating redox active. They have been suggested for reduce the risk of a variety of illnesses such as cancers, liver disease and coronary heart disease. The flavonoids have been also responsible for the characteristic taste and color of tea [6, 7].

Four main catechins including epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) present in the fresh tea leaves [8]. EGCG is the most powerful of the catechins with high antioxidant capacity compare to vitamins C and E [8].

Catechins and polyphenols content of tea are powerful antioxidant agent and may also act as free radical scavenger [7]. In addition, Catechins have many biological potential such as antimutagenic and antitumor activities. They inhibit angiotensin, converting enzyme hyaluronidase, telomerase, α-amylase and glucocyltransferase activity [9-10].
Based on the industrial method, teas are categorized into two main types; non-fermented and fermented. 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 [11]. 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.

*Lippia citriodora* or lemon verbena is a medicinal plant that mostly used as a spice which grows in South America and North Africa and Southern Europe. The leaves of *Lippia citriodora* shows stomachic, and digestive potential, antispasmodic, antipyretic and sedative effects. It used also for the treatment of cold, fever, diarrhoea, indigestion, asthma, flatulence and colic in traditional medicine. According to some study *L. citriodora* contain phenolic acids, iridoids, flavonoids and phenylpropanoids substances with high antioxidant activity. However, there is no available data about antioxidant property of this species in Iran [12].

The aim of recent work was managed to compare the antioxidant properties of different commercial green and black tea brands in Iran with *L. citriodora* as a new beverage. Antioxidant activity of tea infusions was determined based on common synthetic free radical scavenger by 2,2-diphenyl-1-picrylhydrazyl (DPPH), total phenol and flavonoids content.

**MATERIALS AND METHODS**

Tea samples were prepared at central market in Yasuj city Iran. A set of six commercial different brands tea samples consisting four fermented (black), commercial Iranian teas, two, nonfermented (green) tea Iranian brand 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. After extraction 30 min, the infusions were filtered through a filter paper. Name of brand tea: 1= Foman, 2=Tea bag, 3=Shahsavan, 4 =Golabbi, 5= Green 1, 6=Green 2 and 7= Extract

**Determination of total phenol levels:** The total phenolic levels of samples were estimated by Folin-Ciocalteau method with some modifications. Total phenol was expressed as Gallic acid equivalent (GAE) / g tea [13].
Determination of flavonoid levels: The flavonoid level was determined with aluminum chloride (AlCl₃) according to method (Kosalec et al., 2004). The flavonoid values were determined in terms of Rutin equivalents/g tea [14].

Antioxidant activity of Dipheny-picrylhydrazyl (DPPH): The antioxidant activity of extract assessed with little modification. Percent of inhibition was calculated as follow: 
% Inhibition = [(A₀ - A₁)/A₀] × 100 [15].
A₀ is the absorbance of control and A₁ is the absorbance of the plant extracts.

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
The total phenolic concentration of the plant extracts were measured using the Folin-Ciocalteau method by using of Gallic acid as a standard. For determination of flavonoids levels Rutin was use as a standard in aluminum chloride method. The antioxidant activity of the extracts was determined using DPPH / 100 g extract infusion.

Green tea infusions and Lippia citriodora extract had the highest total phenolic concentration. Total phenol content of green tea was reported (14-16 g % GAE). After green teas and Lippia citriodora, The Golabbi, Tea bag, Foman and shahsavan brands were holding maximum amount of total phenol content respectively (Figure 1).

In present study the flavonoid content of extracts ranged from 6.7 to 9.8 g rutin as a standard in 100 gram of extracts. Green tea infusions and Lippia citriodora extract samples were also maximum content and Foman extracts was recorded minimum level of flavonoids content (Figure 2).

DPPH free radical scavenging potential of tea extracts (43.3-54) was demonstrated. Green tea infusion had the highest and Golabbi infusion was the lowest antioxidant activity (53 and 43.3 %) respectively (Figure 3).
Figure 1. Total phenol content of different Iranian tea brands and *Lippia citriodora*. Data are expressed as mean ± SD. Same symbol No Statistically significant difference; different symbol Significant difference at P<0.05.

Figure 2. Total flavonoid content of different Iranian tea brands and *Lippia citriodora*. Data are expressed as mean ± SD. Same symbol No Statistically significant difference; different symbol Significant difference at P<0.05.

Figure 3. Antioxidant acivity Dipheny-picrylhydrazyl(DPPH) of Iranian tea brands and *Lippia citriodor*. Data are expressed as mean ± SD. Same symbol No Statistically significant difference; different symbol Significant difference at P<0.05.
DISCUSSION

According to present research, the most of Iranian brands green tea and *Lippia citriodora* were comparatively higher total phenol and antioxidant activity than that black teas. There was also difference in antioxidant activity and phytochemical components between tea brands. The differences observed between tea brands may be due to a post maturation process where black tea lasts to ferment [13].

Some authors have reported the total phenol level in Australian black tea, with average of 16%, which same the present result [14]. Total phenol content of Iranian tea in present study was similar to previous study in Yasuj Iran (12.6%) [16].

In this study, the total phenol content of green tea extracts was reported higher than that found in most black tea samples. This pattern is also reported in Indian teas varieties (*Camellia sinensis* var. assamica) that have higher total phenol concentration than Chinese variety. In a study by Claudia the total polyphenol content in green tea and black tea were reported 21.02 and 17.62 Gallic acid equivalents (GAE) respectively that parallel to present finding [17].

In recent experiment against most study, there was a weak correlation between total phenols with the antioxidant activities. There was also an inverse correlation between total phenol content of teas with fermentation degree. In present research similar to Atoui, Yokozawa and Satoh experiments, aqueous extracts of green teas contains more total phenol than black teas [18, 19]. Green tea also comprises higher amounts of simple flavonoids – catechins however; black tea contains larger quantity of gallic acid [20].

In study of Khokhar and Magnusdottir, opposite of present study suggested that both aqueous and ethanol extracts black teas contains higher polyphenols concentrations than green teas. However results Manzocco was agree with present finding [21, 22].

Antioxidant activity and total phenol level are two important indicators for evaluating tea quality. They are need for quality control assessment in industrial and imported tea brands also. According to the present results, tea from Yasuj was high quality and *Lippia citriodora* infusion has been comparable antioxidant and phytochemical component with Iranian commercial brands tea.
The antioxidant property of tea associated to phenolic compounds mostly to their hydroxyl groups. Hydroxyl groups are need for antioxidant capacity. These added groups boost donate protons and consequence in antioxidant activity of compounds. Gallic acid is a powerful hydrogen donator. Hence it causes elevated of antioxidant potential in black and green teas. This show why antioxidant potential is elevated in the gallocatechins compounds in green tea.

CONCLUSION
In present work black, green teas and *Lippia citriodora* leaves infusions were demonstrated different level of antioxidant activity via DPPH method. However, green tea mostly revealed the highest antioxidant activity with phenolic compounds.

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REFERENCES