ANTIOXIDANT ACTIVITY OF LIPPIA CITRIODORA AS A NEW BEVERAGE AND SOME IRANIAN BRAND TEA

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ABSTRACT

Introduction and Aim: Tea, *Camellia sinensis* family *theaceae*, is the second beverages consumed daily in the world for centuries. *Lippia citriodora* is a medicinal plant with antioxidant potential which grows in in some parts of world. The aim of present work was estimation of antioxidant activities and phenolic contents of *Lippia citriodora* and compare with commercial green and black tea brands in Iran. Materials and Methods: The samples (2 g) were infused in 1000 ml of boiling water in a flask for 30 Min. Total phenolic content was measured according to the Folin-ciocalteus method. Total antioxidant activity were estimated by different assays such as 2,2-diphenyl-1-picrylhydrazyl radical DPPH, Trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP) and metal chelating. Statistics analysis was estimated by analysis of variance (ANOVA) and followed by post hoc tests. Results: In total phenol, DPPH, TEAC and FRAP methods Green tea and *Lippia citriodora* infusions had maximum and minimum antioxidant activity and phenolic contents respectively. The maximum and minimum chelating activity of *Lippia citriodora* (45%) and Golabbi (32%) were reported respectively. There was a positive correlation between antioxidant activity and total phenol content. Conclusion: *Lippia citriodora* infusions were showed high antioxidant activity via different antioxidant assays compare to Iranian tea infusions. In all methods except metal chelating test, Green tea revealed the highest antioxidant activity with phenolic content. However *Lippia citriodora* infusion had reported with the highest activity in metal chelating potential which comparable with Iranian commercial tea brands in Yasuj Iran.

Key words: Antioxidant activity, total phenols, DPPH, total phenols, TEAC, FRAP, *Lippia citriodora*. 
INTRODUCTION

*Camellia sinensis* family *theaceae*, is the second beverages with high antioxidant activity due to total phenol and flavonoid compounds. *Camellia sinensis* plant present in different forms of tea such as, green, black, oolong and white tea. In Black tea (fermented form) substances are oxidized and catechin was oxidized in controlled condition and theaflavins and the arubigins were generated and mostly used by Western countries [1-4]. In green tea (non-fermented type) with higher antioxidant activity compare to black tea oxidation of green leaf polyphenols was not occurred. Green tea consumed extensively by China and Japan peoples. In green tea oxidation rate in leaves is low and the most of catechins compounds were intact [5].

In Oolong tea production compounds is a partially oxidized whoever in white tea processes the new growth buds and young leaves have been steamed to inactivate polyphenol oxidation and then dried [3].

The flavonoids with potent scavenging potential and chelating redox activity cause reduce the risk of a variety of disease such as solid tumors, liver disease and coronary heart disease risk reduction and also responsible for the characteristic taste and color of tea [6].

Tea catechins and polyphenols are potent free radical scavengers and may also act as antioxidants which may effects on transcription factors and enzyme activities [7,8].

Catechins have many biological potential such as antioxidant, free radicals scavenger, antimutagenic and antitumor activities. They inhibit angiotensin, converting enzyme hyaluronidase, telomerase, α-amylase and glucocyltransferase activity [8-10].

Phenolic compounds in tea infusion reduce the human heart and cancer disease. Antimicrobial potential and anti allergic property of tea reported by some researchers [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*(ORT.) HBK (Verbenaceae) is a medicinal plant that mostly used for stomachic and digestive potential, antispasmodic, antipyretic and sedative effects in ancient medicine in some country. It used also for the treatment of cold, fever diarrhoea, and colic in traditional medicine(12). *It contains* phenolic acids, iridoids, flavonoids and phenylpropanoids.
substances. However, there is no available data about antioxidant activities of this species in yasuj Iran[12].

The aim of recent work was managed to estimation of antioxidant properties of *L. citriodora* as a new beverage by different methods and compare with commercial green and black tea brands in Iran. Total phenol content also was determined according to standard method folin-ciocalteu method.

**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 No. 1. 

% Inhibition = [(A0 - A1)/A0] ×100

% Inhibition = [(A0 - A1)/A0] ×100 [14]. A0 is the absorbance of control and A1 is the absorbance of the plant extracts.

**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].

**Antioxidant activity of Dipheny-picrylhydrazyl (DPPH):** The antioxidant activity of extract assessed with little modification. Percent of inhibition was calculated as follow: 

**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. IC50 or Inhibition concentration in 50% also was determined [15].

**Ferric Reducing Antioxidant Power (FRAP):** The FRAP assay was determined as described by Benzie and Strain methods (16). The results were expressed as micromole Trolox equivalents (TE) per 100 gram of extract.
Metal chelating activity; The chelation of Fe2+ ions by extracts was determined using modified method of Dinis(17).

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. Green tea (No.1) infusion and Lippiacitriodora extract had the highest and lowest total phenolic concentration (16 and 3.45 g % GAE) respectively. Total phenol content (9.53-12.1 g % GAE) content black tea infusions was reported less than green tea (Figure 1). In total phenol, DPPH, TEAC and FRAP methods Green tea and Lippiacitriodora infusions had maximum and minimum antioxidant activity and phenolic contents respectively (Figure 1-4). DPPH free radical scavenging potential of tea extracts (30-56%) was measured. Green teas and Lippiacitriodora infusion had the highest and lowest antioxidant activity by DPPH method (56 and 30%) was reported respectively (Figure 2). The antioxidant activities of the extracts were determined as trolox equivalents (mmoltrolox/g extract) using TEAC and DPPH, and FRAP as mmole Fe / 100 g extract infusion. The highest antioxidant potential was observed with extracts in the TEAC assay (84%) followed by DPPH test (53%) and Iron chelating (45%) (Figure 3). The antioxidant activity by TEAC in tea infusions (26-84% inhibition) was measured. Similarly, Green tea (No.1) infusion and Lippiacitriodora extract had the highest and lowest antioxidant activity by TEAC method (84 and 26%) respectively (Figure 3).

The antioxidant activities varied from 343 to 450 mmol Fe2/g extract by the FRAP assay (Figure 4). Similar to TEAC and total phenol assays green tea (No.1) infusion and Lippiacitriodora extract had the highest and lowest level of antioxidant activity in FRAP method Figure 4. The maximum and minimum chelating activity (% of inhibition) of extracts Lippiacitriodora (45%) and Golabbi (32%) were reported respectively(Figure 5). In present experiment a high relationship with correlation coefficients ($r^2$) =0.6932 was reported between the total phenol content and antioxidant activities by ABTS assay (Figure 6). However a weak correlation was observed between total phenol content and FRAP assay (Figure 7).
Figure 1: Total phenol content of different Iranian tea brands and *Lippia citriodora*. Same symbols no statistically significant difference; different symbols significant difference at P<0.05. Data are expressed as mean ± SD.

Figure 2: Antioxidant activity of diphenyl-picrylhydrazyl (DPPH) of Iranian tea brands and *Lippia citriodora*. Same symbols no statistically significant difference; different symbols significant difference at P<0.05. Data are expressed as mean ± SD.

Figure 3: Antioxidant activity of Trolox equivalent antioxidant activity (TEAC) of Iranian tea brands and *Lippia citriodora*. Same symbols no statistically significant difference; different symbols significant difference at P<0.05. Data are expressed as mean ± SD.
Figure 4: Antioxidant activity of Ferric Reducing Antioxidant Power (FRAP) of Iranian tea brands and *Lippia cithriodora*. Same symbols no statistically significant difference; different symbols significant difference at $P<0.05$. Data are expressed as mean ± SD.

Figure 5: Metal chelating of Iranian tea brands and *Lippia cithriodora*. Same symbols no statistically significant difference; different symbols significant difference at $P<0.05$. Data are expressed as mean ± SD.

Figure 6: Correlation of total phenol content with antioxidant activity by Trolox equivalent antioxidant activity (TEAC method in Iranian tea brands and *Lippia cithriodora*).
Figure 7: Correlation of total phenol content with antioxidant activity by Ferric Reducing Antioxidant Power (FRAP) method in Iranian tea brands and Lippia citriodora.

DISCUSSION

In recent study, the most of Iranian brands tea infusions were comparatively higher total phenol and antioxidant potential reported than that Lippia citriodora leaves infusion. However, metal chelating activity of Lippia citriodora infusion was reported with the maximum activity and its flavonoids content was high level compared to tea brands.

In present study, there was also difference in antioxidant activity and phytochemical components between tea brands. The differences observed between teabrand may be due to a post maturation process where black tea lasts to ferment [18]. Some authors have reported the total phenol level in Australian black tea, with an average of 16%, which relatively higher than the average reported in present study (11.2%), [19]. Total phenol content of Iranian tea in present study was similar to previous study in Yasuj Iran (12.6%) [20].

In this study, the total phenol content of green tea extracts was reported higher than that found in most black tea samples. Usually, Indian teas varieties (Camellia sinensis var. assamica) have higher total phenol concentration than Chinese variety (Camellia sinensis var. sinensis), [21, 22].

Antioxidant activity and total phenol level is 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 were need for antioxidant capacity. These added groups boost donate protons and consequence in antioxidant activity of compounds. Gallic acid is a powerful hydrogen donator to DPPH test hene, presents elevated antioxidant potential in black and green teas. This show why antioxidant potential is elevated in the gallocatechins compounds in green tea [23].

In this research similar to other studies, Green tea had considerably ($p < 0.05$) higher antioxidant capacity compared to black tea [24]. There was significant difference in the antioxidant potential of green and black tea by DPPH and TEAC assays They have prevented the cell from free radical injury by decreasing of transition metal ions (25). Different plant extracts were demonstrated to be good chelators and association present between total phenols, flavonoids and chelating potential.

*Lippia citriodora* infusion presented maximum chelating activity in present study. Metal chelating capacity infusions may be due to presence of their total phenol compounds and antioxidant activities[26]. Metal chelating is very impotent for iron overload states. In such condition metal chelators in plant sources can bind with iron ions in plasma or biological systems for remove or decrease of iron.

Removal of iron in diverse forms such as soluble and stable complexes was released in urine and feces by iron chelators in chelation practice. Therefore, plasma iron concentration and iron overload complications was reduced and increased quality of life. In thalassemia and Alzheimer’s disease iron toxicity is a main crisis. Therefore, iron chelation is a beneficial policy for survival [27].

Some plant extracts with antioxidant activity have exhibited metal chelating potential and inhibition of free radical injury by decreasing of transition metal ions [25]. For evaluation of this property determination of iron chelating is essential step [28]. Therefore, use of *Lippia citriodora* leaves infusion is important in iron overload states such as thalassemia and Alzheimer’s disease.

**CONCLUSION**

In present work black, green teas and *Lippia citriodora* leaves infusion were demonstrated different level of antioxidant activity via DPPH, TEAC, FRAP and metal chelating methods.
However, green tea mostly revealed the highest antioxidant activity with phenolic compounds. *Lippia citriodora* infusion with high flavonoid content showed the highest activity in metal chelating potential.

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REFERENCES


