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Research Article

A Comparative Study of the Total Phenolic, Total Antioxidant, and Ascorbic Acid Contents of the *Cistus criticus*, Fermented Rooibos, and Green Tea Infusions

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ABSTRACT

Plants and herbs have been used as a traditional medicine due to their phytochemical contents since ancient times. Most plants are known for their various physiological advantages thanks to their bioactive compounds. These plant-sourced herbs are mostly consumed as tea infusions. Noteworthy, tea is the second most consumed beverage throughout the world. Due to the natural bioactive content of the tea beverages they are considered as functional foods. Functional foods either prevent or avoid the progression of diseases and they are mainly rich in antioxidants and polyphenols. Functionalized food consumption has increasing trend not only due to disease prevention/delaying but also due to other health and well-being concepts. This study was motivated with the aim of comparing the brewing technique (hot and cold) for the total phenolic content, antioxidant activity and ascorbic acid levels of the *Cistus criticus*, fermented rooibos, and green tea infusions. Results obtained from this investigation will illustrate a comparative image between the selected tea infusios of hot and cold brews. Findings of this research Results suggested suggests that hot brewing is more efficient in terms of for total phenolic content while the opposite for the total antioxidant activity ($P < 0.05$). On the other hand, comparison between the three selected herbal tea samples, Also, *Cistus criticus* infusions showed the highest content of total phenolic compounds, while green tea demonstrated the highest antioxidant activity for hot and cold infusions. Ascorbic acid levels of these 3 herbal tea samples did not show any significant difference ($P > 0.05$). Noteworthy, this study cannot suggest any correlation between the total phenolic content and total antioxidant activities for the water extracted hot and cold infusions of *Cistus criticus*, fermented rooibos, and green tea samples.

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Introduction

Tea is the general term for the drink made from the leaf, bud or the flowers of the plant. It is the second most consumed beverage throughout the world [1]. Historically, leaves, buds, and flowers of the plants have been used for more than 4000 years as traditional medicine [2]. In addition to black and unfermented black (green) tea, various herbal teas are becoming more and more popular due to their functional properties. Previous studies and ancient scripts show that tea is rich in vital nutrients and healthy components such as phenolic compounds which shows antioxidant activity due to flavonoids [3]. Functional advantages and species-dependent properties of the drinks of the tea can be classified as: pharmacological [4, 5], toxicology [6], anti-inflammatory and anti-oxidative [7–10], and metal-chelating [11]. Despite traditional teas such as black tea [12–14], and green tea [15–17] there are many more functional herb that are consumed as tea for example: lemongrass tea [18], chamomile tea [19, 20], or mate tea [21, 22] due to their functional properties.

In order to limit or eliminate the cellular damage of the reactive oxygen, the human body greatly depends on the anti-oxidative body processes as well as antioxidant food sources. Also, for most of the bodily functions and operations, it is necessary to evoke the inflammatory process that occurs due to the noxious agents (e.g. infections). As mentioned earlier tea is a traditional ancient beverage that is used as a medicine mainly due to its flavonoid content. Flavonoids are phenolic compounds of the plants with a wide variety of structures and biological property which is addressed as the responsible body of the health benefits of those foods [1]. Previously examined tea sources and their flavonoids illustrated that they are responsible for the cell and tissue protection against the free oxygen radicals. Cell and tissue damage usually end up with life-threatening diseases such as cardiovascular diseases, cancer and etc. Naturally human body is capable of developing this free oxygen radical defense system; however, in some cases, it may not be sufficient solely. Under these conditions physicians usually suggests diet-derived sources of antioxidants to protect from cell and tissue damage of the patient. Tea is one of the main diet-derived external sources of cell protector and due to its availability, economic advantage, and easy/favorable consumption.

In this study, cistus (*Cistus criticus*) tea, fermented rooibos tea, and green tea were infused in hot and cold brewing techniques and tea samples were examined for their total phenolic, antioxidant and ascorbic acid content. This study aimed to illustrate the basic functionality of these herbs with different brewing temperatures. Additionally, comparison of the brewing temperature, it will be possible to suggest best possible brewing conditions to the consumer directly for the optimum functionality as well as findings will be useful for further food science experiments to obtain the bioavailability and the possible component extraction opportunities.

Materials and Methods

Materials

Herbal tea samples were infused as follows. Cistus tea (CT) was obtained from the Cistus leaves which were collected from the Alanya-Gazipaşa region of Antalya (Turkey). These leaves were washed and dried by using conventional oven-drying method for 5h at 50°C (Memmert UN55) [23]. The dried leaves were then grounded in a mill (Warring 8011 Blender). Green tea (GT) (Çaykur Her Dem Yeşil) and fermented rooibos tea (FR) (Aktar Diyarı Red Rooibos) were obtained commercially from the local store in Alanya/ Antalya. Leaf samples were used after no later than 1 month of packing. Dried leaf samples were stored at -20°C before use. All chemicals were purchased from Carlo-Erba Chemical Company (Milan, Italy). Chemicals were in analytical grade.

Brewing of Tea

Tea samples were prepared by 2 different methods; hot and cold brewing according to the methods obtained from Lin, et al., (2008). For the hot brewing method; 57 g of leaf sample was added to 1000 ml distilled hot water (95°C) for 20 minutes and filtered through Whatmann No.1 filter paper. On the other hand, for the cold brewing method; same amount of leaf was added to 1000 ml distilled cold water (4°C) and stored at the room temperature for 24 hours and filtered through Whatmann No.1 filter paper. For both brewing methods the filtrate was cooled to room

temperature prior use and tea samples were prepared before each experiment. The tea samples were assessed for their total phenolic content, antioxidant activity, and ascorbic acid content in order to provide a comparative approach. Before the analysis tea samples were centrifuged at 4000 rpm for 10 minutes [25].

Total Phenolic Content Analysis

Total phenolic content was measured according to the method applied by Singleton et al., (1999) and Bramati et al., (2003). 1 ml of the centrifuged extract was added to 9 ml of distilled water and 0.25 ml of this mixture were added to 1 ml of distilled water and mixed with 0.25 ml of Folin-Ciocalteu reagent. After 6 minutes of storage mixture were added to 2.5 ml (7%) sodium carbonate and 2 ml of distilled water. After mixing with vortex, the mixture was stored at the darkroom for 90 minutes and absorbance value was measured against control at 760 nm using UV-Visible spectrophotometer (Shimadzu, UV-1280). Absorbance values were then used for calculating the total phenolic content by using the Gallic acid standard curve (y=4696.4x-0.0117, R^2=0.9984).

DPPH Radical Scavenging Capacity

The total antioxidant content of the tea samples was measured by using DPPH radical scavenging method obtained from Brand-Williams et al., (1995). According to this method 100 µl of centrifuged tea sample was mixed with 3.9 ml of (0.1 mM) DPPH-methanol solution. After 2 minutes of vortex mixing the extracts were stored in the darkroom for 30 minutes. Following the 30 minutes reaction, absorbance values of the solutions were measured at 515 nm with UV-Visible spectrophotometer against blank solution. % inhibition values were calculated as;

$$\%\, Inhibition = [(A_0 - A_1)/A_0] \times 100$$

where; A_0 is the absorbance of the control and A_1 is the absorbance of the test sample.

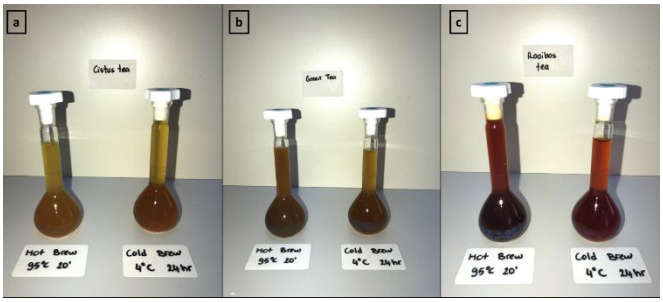
Ascorbic Acid Content

Ascorbic acid content was determined with the spectrophotometric method as applied by Murathan, (2017). According to this method, 100 µl of centrifuged tea sample was mixed with 400 µl oxalic acid (0.4%) and 4.5 mL 2,6-dichlorophenolindophenol (30 ppm) solution which followed by vortex mixing. Absorbance values of these solutions were measured immediately at 520 nm by using UV-Visible spectrophotometer. Absorbance values were then used for calculating the total ascorbic acid content by using the ascorbic acid standard curve (y=-0,0004x+0.3236, R^2=0.9985).

All experiments were done in triplicate. Statistical analyses were conducted using XLSTAT (v.2014 and Prism 6.0) statistical software (Microsoft, Mountain View, CA). For data analysis, mean, median, standard deviation and coefficient of determination (R^2) values were calculated. Differences between the groups were calculated with Duncan's test, and results were considered to be statistically significant with a 95% confidence level ($P<0.05$).

Results

CT, GT and FR samples were hot and cold-brewed in order to observe the effect of the brewing temperatures on the total phenolic (TPC), total antioxidant activity (TAC) and ascorbic acid (AsA) content. A visual illustration of the hot and cold-brewed samples can be viewed from Figure 1.



Hata! Başvuru kaynağı bulunamadı. *Visual illustration of hot and cold-brewed tea samples for (a) cistus tea, (b) green tea, and (c) rooibos tea.*

Table 1 shows the TPC, TAC, and AsA of the CT, GT, and FR for theşr hot and cold infusions.

Table 1 TPC, TAC, and AsA results of the CT, GT, FR samples. All experiments were done in triplicate and statistical analyses were made at 95 % significance level. Letter indices represent different groups.

		Total Phenolic Content (Gallic acid equivalents (GAE) in mg/g)	Total Antioxidant (% DPPH Radical Scavenging capacity, %Inhibition)	Ascorbic Acid Content (Ascorbic acid equivalents in mg/100g dry weight)
Cistus Tea*	Hot Brewing	17.283±0.03[a]	85.79±0.30[f]	0.3232±0.01[l]
	Cold Brewing	8.754±0.01[b]	86.19±0.10[g]	0.3233±0.01[l]
Green Tea*	Hot Brewing	10.867±0.01[c]	85.95±0. 20[h]	0.3236±0.02[l]
	Cold Brewing	10.480±0.01[c]	90.68±0. 10[i]	0.3234±0.01[l]
Fermented Rooibos Tea*	Hot Brewing	5.359±0.01[d]	73.05±0.20[j]	0.3235±0.005[l]
	Cold Brewing	1.587±0.04[e]	80.15±0.10[k]	0.3234±0.001[l]

* Values are means of triplicate determinations ± standard deviation. Values within a row with different letters are significantly different (P <0.05)

On the other hand, TPC results of the 3 herbal tea sample can be seen in Figure 2.

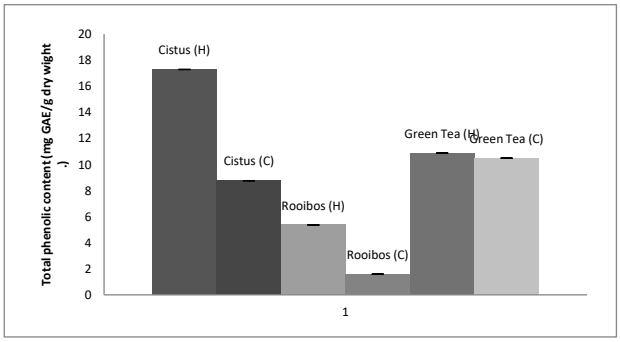


Figure 1 Total phenolic content of the hot and cold-brewed CT, GT, and FR. (C) represents cold brewing, whereas (H) represents hot brewing technique.

The DPPH method for total antioxidant activity determination is one of the commonly used methods. The method depends on the measurement of the deep violet color by absorbance disappearance as a result of the stoichiometric discoloration due to the decrease in free radicals. The DPPH radical scavenging activity of water extracted CT, GT, and RT at 0.057 g/ml concentration was 85.8 %, 86.0 %, and 73.1 % respectively for hot brewing technique. Meanwhile, the DPPH radical scavenging capacities of the CT, GT, and RT for cold-brewed samples were 86.2 %, 90.7 %, and 80.1 % respectively.

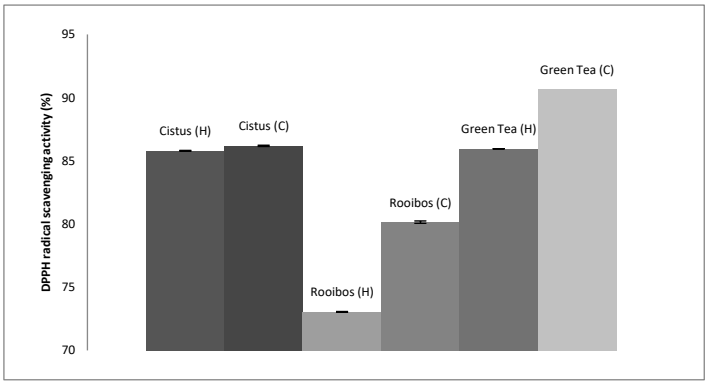


Figure 2 %DPPH radical scavenging activity of the hot and cold-brewed CT, GT, and FR. (C) represents cold brewing, whereas (H) represents hot brewing technique.

Discussion

As illustrated at the Figure 1 Comparison between the 3 herbal tea samples was aimed to be determined which will be useful for the daily consumption of the consumers as well as public health. As already mentioned by Lin, Liu, and Mau (2008), cold water extraction was less effective than hot water extraction for the total phenolic content.

TPC Content

Phenolic compounds are secondary plant metabolites that are vastly available in most of the fruits and vegetables [29]. The unique and significant features of the phenolic compounds derive from their wide range of biological and pharmacological activities [30]. In the present study TPC results of the 3 herbal tea sample can be seen in Figure 2. For GT, RT, and CT the TPC content was between 17.283 – 1.587 as Gallic acid equivalents (GAE) in mg/g dry weight. Statistically all of the tea samples have shown significant differences for the hot and cold brewing techniques except for the green tea sample. Specifically, hot brewing or thermal application increases the phenolic content of the drink which was supported in the literature [24, 31–33].

In this study, the phenolic content of hot infusions ranged from 17.283±0.03 to 5.359±0.01 GAE mg/g dry weight, whereas that of cold infusions ranged from 10.480±0.01 to 1.587±0.04 GAE mg/g dry weight. The highest

phenolic content levels were observed in hot infusions of CT, whereas lowest total phenolic content was observed in cold infusions of FR sample. As can be seen from Figure 2, CT tea shows significantly higher levels of TPC compared to RT and GT. In the literature, extracting of the phenolic compounds were mostly achieved by either ethanol or methanol solvents. However, in this study water was selected as the solvent. Few literature findings with water dissolved herbal tea are; hot extraction of fermented rooibos tea 27.09% of total polyphenols (GAE g/g) [34], hot water extracted green tea 112.3 mg/g GAE [25]. Meanwhile, ethanol or methanol extracted samples were determined to be; ethanol extracted CT about 11.90 mg/g GAE dry weight [29, 35], methanol extracted CT about 18 mg GAE/g of extract [35], and ethanol extract CT were 41.73-98.69 mg GAE/g dry weight [36]. These findings of CT and RT are mostly compatible with present findings; however, it is also significant to highlight the solvent effect which is expected to show decreased level of compound transfer when water is the solvent.

DPPH Radical Scavenging Capacity

DPPH radical scavenging capacities of the herbs and plants have been previously considered by various researchers [37–39]. Two brewing methods were statistically different (P <0.05) and cold brewing techniques found to be more efficient in terms of antioxidant compounds transfer to the aqueous solution. On the other hand the DPPH radical inhibition value was higher for GT samples (P <0.05) compared to other tea infusions. In the literature water extracted FR tea was observed to have 2180 μmol/g extract) for the DPPH scavenging ability [34], whereas for methanol extracted CT 87.72 % inhibition were determined.

Ascorbic Acid Content

Ascorbic acid levels of the tea infusions were between 0.3232 and 0.3236 mg/100g dry weight. Statistical observations on the brewing temperature and herbal tea samples did not show any significant difference. This finding may show that more analytical methods might be required in order to observe the differences between the brewing temperatures of the herbs in terms of ascorbic acid levels.

Conclusions

Using plants and herbs as a traditional medicine due to their phytochemical contents is an approach used since ancient times. Most plants are known for their various physiological advantages thanks to their bioactive compounds. Ancient treating effects of foods were sourced due to their curing effect on the abundance of reactive oxygen species and free radicals. These foods which either prevent or avoid the progression of some diseases are mainly rich in antioxidants and polyphenols, which are usually defined as functional foods. Functionalized food consumption has increasing trend not only due to disease prevention/delaying but also due to other health and well-being concepts. Adding a function without any ingredient addition (natural) is one of the most attractive topics of the functional foods. Antioxidant properties and polyphenols of the foods are effective especially on the mechanisms of free radical scavenging, hydrogen donation, singlet oxygen quenching and being a substrate for oxidation reactions [40, 41]. Phenolic and antioxidant investigation of the herbal sources have been widely studied in the literature, especially for the selected three herbal tea samples [42–45]. This study was motivated with the aim of comparing mostly referred tea samples with each other for the basic functional ingredients (TPC, TAC, and AsA). Hence, water extracted material was analyzed in order to represent the real consumption conditions. Beyond the traditional use of the selected herbal tea samples, results illustrated that intermediate value-added product replacement is possible with the tea samples. The present study showed that the temperature of the water extraction on the content basis is significant for the TAC and TPC. Several factors contribute to the different levels of quantitative observations, which could be listed as; stem-leaf ratio (of the plant), particle size of the material (which affects the mass transfer), solvent-to-solid ratio during extraction and infusion.

As illustrated visually and analytically, the temperature of brewing has significant effect on water-soluble component extraction from the tea-leaves. The particle size of the leaves, extraction temperature and duration is critical for the components which have functional significance such as phenolic [24, 46]. Hence, considering the process for home use and

industrial applications for the tea leave brewing, hot extraction brewing has shown the most efficient process, especially for TPC. One of the main aims of this study was observing the one cup serving product properties in terms of significant components such as antioxidants and phenolic contents which requires using water as the solvent. As mentioned earlier at the year of 2012 by Joubert and de Beer, there is missing information in the literature about the one-cup content of the functional tea brews, as well as comparative approach to those tea samples. This study illustrated that hot brewing is more effective in terms of TPC whereas opposite is preferable for TAC. Additionally cistus tea seems to show higher total phenolic content and green tea with highest total antioxidant activity between the tested 3 herbal tea samples regardless the brewing temperature

On the other hand, we usually expect that high polyphenol content to exhibit high total antioxidant activity. However, this study cannot suggest any correlation between the TPC and the TAC content of the water extracted hot and cold infusions of CT, GT, and FR tea samples.

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