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# Phytoconstituents and antioxidant properties among commercial tea (*Camellia sinensis* L.) clones of Iran

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ABSTRACT

*Background:* Tea (*Camellia sinensis*), a well-known beverage is consumed frequently worldwide due to its high antioxidant properties. The present study determines the amount of phytochemicals and antioxidant activities among 12 high yielding tea clones cultivated in Iran.
*Results:* Among the 12 clones studied, tea clone Iran 100 had the highest total phenolic content and total flavonoid content with values of 8.44 ± 1.03 mg gallic acid equivalents per gram dry weight and 4.50 ± 0.16 mg rutin equivalents per gram dry weight respectively. High performance Liquid Chromatography (HPLC) analysis of phenolics and flavonoids in 12 clones revealed the presence of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epigallocatechin-gallate, (−)-epicatechingallate, gallic acid and caffeine. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay showed the existence of variation in the antioxidant activity ranging from 22.67 to 65.36%. The highest antioxidant activity with $IC_{50}$ value of 218.24 μg/mL was observed in the leaf extract of the clone Iran 100, while the lowest was found in the clone Iran 482 with $IC_{50}$ value of 234.44 μg/mL. The antioxidant activity had a positive correlation with total phenolic content, total flavonoid content, (−)-epigallocatechin-gallate, (−)-epicatechingallate and caffeine ($0.59 \le r \le 0.97$, $P < 0.05$).
*Conclusion:* From the study it can be concluded that the clone Iran 100 has a superior quality compared to any other clones studied due to occurrence of more phenolic compounds and a greater antioxidant activity. Hence, we recommend the use of tea clone Iran 100 for commercial planting.


## 1. Introduction

Antioxidants are chemical substances used for treating various human diseases related to heart, lungs, kidney, muscle, brain and helps to control aging process. Antioxidants effectively function in human body by inhibiting or delaying the formation of free radicals and lipid peroxidation that are mainly responsible for many human diseases and aging process [1,2]. Plant based natural compounds have been accounted for a wide range of biological properties such as antioxidant, anti-inflammatory and antimicrobial activities [3,4,5]. The presence of different phytochemicals such as ascorbic acid, tocopherols, carotenoids, and polyphenolic compounds and their combined activities result in the total antioxidant activity of a plant. However, polyphenolic compounds from plants appear to have the greatest antioxidant potential and could be the most beneficial antioxidants [6]. Many of these common antioxidant compounds are found in fruits and vegetables. Plants are known to possess polyphenolic compounds such as flavonoids, and other phytochemicals such as carotenoids [7]. Karimi et al. [1] proposed that plant fruits contain a variety of (poly) phenolics and (poly) phenolic derivative compounds and many of these compounds could be potential antioxidant sources. Tea (*Camellia sinensis*) plants belonging to Theacea family are known to contain higher antioxidant compounds. Tea is been one of the widely consumed beverages in the world. Tea is a perennial evergreen plant that requires humid and warm environmental conditions. Native to Southeast Asia, tea has been planted widely in tropical and subtropical areas. Near the equator, it ranges up to nearly 2000 m elevation. It thrives well on well-drained acidic soils (pH 4.5–6.0) and requires temperatures ranging from 13°C to 30°C with an annual rainfall of about 120 cm or more. The quality and uniqueness of each tea brand depends on many parameters including the growing seasons, geographic regions, processing and fermentation methods. Iran is one of the 13 major tea producers in the world. Two evergreen regions in the Northern region of Iran are Gulian and Mazandaran (36°/31 to 37°/25 N, and 49°/15 to 51°/15 E) and these are the important regions contributing to tea production. This land stretches to about 34,000 ha and is used for the production of tea [8]. There are many types of tea, all classified based

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on how they are processed. Green tea and black tea are two of the major commercial types of tea [9]. Among the daily food and beverage products, tea is very rich in flavonoid compounds mainly catechins which mainly accumulate in growing tea leaves. Catechins are naturally occurring polyphenols found in tea, red wine, chocolates and many fruits. They belong to the flavonoid group and are considered as flavan-3-ols. Common catechins found in tea are (−)epigallocatechin (EGC), (−) epigallocatechin-3-gallate (EGCG), (−)epicatechin-3-gallate (ECG) and (−)epicatechin (EC).

Tea is an important beverage in Iran and to date the demand for tea is increasing. However, most tea plantations are old resulting in low productivity. It is therefore, necessary to replace the existing plantation with elite clones with the best quality. Presently, there are more than 20 registered high yielding clones in Iran such as 102, 449, 219 and clone Iran 100. Selection of elite planting material for commercial plantation from the above mentioned clones can be better achieved based on chemical profiling and studying their biological activities. Therefore, the present study was aimed at characterizing the phytochemical constituents and antioxidant activity in different tea clones of Iran to compare their quality attributes.

## 2. Materials and methods

### 2.1. Plant material

Leaves from 12 different tea clones (*C. sinensis* (L.) O. Kuntze) (Fig. 1) namely Fashalam, 100, 102, 178, 218, 219, 223, 404, 437, 449, 482 and 1102 were obtained from the Tea Research Institute of Iran with the GPS location of 37° 12′ 33″ N latitude and 50° 0′ 2″ E longitude.

### 2.2. Preparation of extracts

The extraction procedure was carried out by using the method of Crozier et al. [10]. Briefly, freeze-dried leaves (2 g) each from 12 tea clones were weighed and added to 100 mL conical flask contained with 40 mL of 80% (v/v) methanol [10]. Later, 10 mL of 6 M HCl was added and the mixture was refluxed for 2 h at 90°C. The mixture was filtered by using Whatman No. 1 filter paper (Whatman, England) and the filtrate was evaporated to dryness using a vacuumed Rotary Evaporator (Buchii, Switzerland). The known quantity of dried crude extract was dissolved in methanol and stored at −20°C for future studies.

### 2.3. Determination of total phenolic content

Folin–Ciocalteu's reagent method was used to determine the amount of total phenolic compounds in the extract [11] and the results obtained were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW).

### 2.4. Determination of total flavonoid content

Total flavonoid compounds was measured using the aluminum chloride colorimetric assay described by Ismail [12]. Total flavonoid compound of extracts were expressed as mg rutin equivalent (RE)/g DW.

### 2.5. Analysis of phenolic and flavonoid compounds by RP-HPLC

High Performance Liquid Chromatography (HPLC) grade methanol and acetonitrile were procured from Merck Chemicals (Darmstadt, Germany). Five flavonoid standards; (−)-EC, (+)-catechin (C), EGC, EGCG, ECG were obtained from Chromadex (Irvine, CA, USA). Gallic acid and trifluoroacetic acid (TFA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Caffeine was supplied by Wako (Japan). Double distilled water was obtained from a Mili-Q purification system supplied by Milipore Laboratory (Bedford, MS, USA).

Both qualitative and quantitative analysis of *C. sinensis* extracts were analyzed on Waters 2695 Alliance HPLC System equipped with 996 photodiode array detector (PDA) (Waters, MA, USA). A C18 Synergi column (250 × 4.6 mm, i.d., 4 μm, phenomenex, CA, USA) was used. The column temperature was maintained at 40°C. A binary solvent system of 0.005% TFA in deionized water (solvent A) and 0.005% TFA in acetonitrile (solvent B) was developed as follow: 0–5 min, 5% B; 5–10 min, 5–10% B; 10–32 min, 10–30% B; 32–35 min, 30–95% B and finally washing the column with 95% B for 2 min and reconditioning with 5% B isocratic for 3 min. The mobile phase was degassed before HPLC injection. Flow rate was set at 1.00 mL/min. Signal was monitored at 280 nm. Data acquisition was performed using Waters Empower 2 software (Waters, MA, USA).

All standard solutions were prepared in methanol. Calibration curves were obtained for C, EGC, EGCG, CAF and ECG using a series of standard solutions over a four point concentrations (25–500 μg/mL). All calibration curves were linear over the concentration ranges tested with correlation coefficients ≥0.998.

### 2.6. Determination of total antioxidant activity by DPPH free radical scavenging assay

Total antioxidant property of the extract was determined by using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay as described by Yen et al. [13]. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity. The free


**Fig. 1.** The leaves of twelve different tea clones (*Camellia sinensis* (L.) O. Kuntze) used in the present experiment for the determination of antioxidants.

radical scavenging activities of the tested samples were expressed as percentage of inhibition and were calculated according to [Equation 1]:

$$\%\ \text{inhibition of DPPH activity} = [(A_0 - A_1) / A_0] \times 100 \quad \text{[Equation 1]}$$

where $A_0$ is the absorbance value of the blank sample or control reaction and $A_1$ is the absorbance value of the test sample. A curve of percent inhibition or scavenging effect against sample concentrations was plotted and the concentration of the sample required for 50% inhibition ($IC_{50}$) was determined. The value for each of the test sample was presented as inhibition curve at 50% or $IC_{50}$.

## 3. Results and discussion

### 3.1. Total phenolics and flavonoids content

The plant secondary metabolites including flavonoids and phenolics are known to improve human health by controlling various cellular pathways and helps to provide antioxidant effects. There is an increasing interest toward research on flavonoids occurring in various sources of dietary supplements due to their multipurpose health benefits such as free-radical scavenging property, anticancer activity, prevention of coronary heart disease and antiviral properties [1,6]. Hence, there is a need to understand the chemical structures and biological functions of flavonoids to be used as a potential therapeutic agents as well as to predict or control the quality of food [14]. Among the day to day dietary food supplements, tea is very frequently consumed all over the world. Tea is very rich in polyphenols that acts as antioxidant agent to detoxify free radicals and prevents cell damages in the body.

In the present study, the result of the amount of total phenolics and flavonoids of 12 clones of tea showed significant differences. Among the 12 clones studied, tea clone Iran 100 had a higher phenolic and flavonoid content with a value of 8.44 ± 1.03 mg GAE/g DW and 5.40 ± 0.72 mg RE/g DW respectively, when compared to other tea clones (Table 1). The next best clone to contain higher phytochemicals was Iran 404 with 8.26 ± 1.09 mg GAE/g DW of phenolic content and 5.28 ± 0.65 mg/g RE DW of flavonoid content.

### 3.2. RP-HPLC-PDA analysis

The HPLC absorbance profile of 12 *C. sinensis* clones analyzed by reversed phase HPLC-PDA is presented in Fig. 2. Five types of flavonoids; C, EGCG, EGC, EGCG, ECG were identified together with gallic acid and caffeine in all 12 samples based on the comparison of the retention time and absorption spectra of the reference standard with those of the tea clones. This identification is in good agreement with the tea chemical composition widely described elsewhere [15,16]. In the present study, CAF, EGCG and ECG were further quantified since the content of these compounds are associated with the quality and taste of tea as well as to correlate the major components of the clone extracts with the free radical-scavenging properties.

The mean value of EGCG, ECG and CAF in the 12 tea clones analyzed in this study is given in Table 2. A wide variability for EGCG, ECG and CAF content was noted among the tea clones. The content of EGCG varied between 1.60 ± 0.18 mg/g and 4.90 ± 0.19 mg/g and the ranked order of the mean values was 178 > 1102 > 100 > 404 > 223 > 219 > 102 > Fashalam > 218 > 482 > 437 > 449. ECG levels varied between 0.59 ± 0.07 mg/g to 1.55 ± 0.14 mg/g, while the levels of CAF ranged from 1.52 ± 0.08 mg/g to 3.78 ± 0.10 mg/g and the ranked order of the mean value of ECG and CAF was 100 > 219 > 178 > 482 > 218 > 102 > 404 > 437 > 1102 > 223 > Fashalam > 449 and 100 > 218 > 219 > 178 > 437 > 404 > 449 > 482 > 102 > 1102 > Fashalam > 223 respectively. EGCG was identified as the most abundant component in all clones except clone 218, 219, 437 and 449. These four clones had a higher amount of CAF suggesting their high quality attributes. Moreover, previous study also confirmed the excellent biological activities of CAF [17].

### 3.3. Antioxidant assay of tea leaves extracts

Antioxidants are chemical substances that inhibit the oxidation of other molecules and protect cells from the damages caused by free radicals in the human body. These antioxidants are found in plant foods including fruits, vegetables and other dietary supplements. These antioxidants can alleviate and prevent various diseases related to cardiovascular system, heart, brain, liver, kidney and cancer and it helps to retard the aging process [17,18]. Antioxidant activities are determined by using the DPPH method due to radical scavenging in a relatively short time in comparison to other methods [19]. The possible antioxidant effect of radical scavenging activity is due to the ability of DPPH to donate hydrogen ions. Hence, DPPH is a free radical which readily accepts either electron or hydrogen radical and becomes more stable molecule [20]. The result of the DPPH scavenging assay of the 12 clones of tea at the concentration of 250 μg/mL is shown in Table 3. Among all clones tested, the clone Iran 100 had exhibited higher antioxidative activities (65.4%). However the standards, butylated hydroxytoluene (BHT) (99.2%) and α-tocopherol (99.7%) showed the highest antioxidant activities when compared to all samples. The $IC_{50}$ (Inhibition concentration at 50%) of the leaf extracts of 12 clones of tea, BHT and α-tocopherol on scavenging activity of free radicals are presented in Table 4. The $IC_{50}$ of α-tocopherol and BHT were 60.39 and 89.77 μg/mL respectively. Meanwhile, the highest antioxidant activity was exhibited by leaf extract of clone 100 ($IC_{50}$: 218.25 μg/mL), and the lowest was recorded from clone 482 ($IC_{50}$: 234.44 μg/mL). Phenolic and flavonoid compounds are known to be very important plant secondary metabolites due to their various biological activities and pharmaceutical properties especially they serve as a better antioxidants [21]. Many phenolic and flavonoid compounds have been reported for their beneficial effects such as antioxidant property, anticancer, antimicrobial and anti-inflammatory activities in a greater or lesser extent [22].

### 3.4. Correlation analysis

*C. sinensis* has been reported to have as many as 700 chemical compounds and could be classified into polyphenols (major constituents), polysaccharides, proteins, methylxanthines (i.e.: CAF), minerals, amino acids, lipids and organic acids [23]. The most important group of polyphenol in tea is the catechins because of the ability to prevent oxidative modifications of cellular lipids, proteins, and nucleic acid by multidirectional antioxidant actions [24]. Besides

**Table 1**
Total phenolic and flavonoid content obtained from extract of tea leaves from 12 clones.

| Clone | Total phenolic content[1] | Total flavonoid content[2] |
|---|---|---|
| Fashalam | $6.55 \pm 0.33^{cd}$ | $3.70 \pm 0.16^{bc}$ |
| 100 | $8.44 \pm 1.03^{a}$ | $5.40 \pm 0.72^{a}$ |
| 102 | $6.89 \pm 0.59^{cd}$ | $4.85 \pm 0.54^{b}$ |
| 178 | $7.39 \pm 0.35^{bc}$ | $4.46 \pm 0.71^{bc}$ |
| 218 | $7.05 \pm 0.56^{bcd}$ | $3.68 \pm 0.52^{c}$ |
| 219 | $7.28 \pm 0.27^{bcd}$ | $4.35 \pm 0.59^{bc}$ |
| 223 | $7.92 \pm 0.43^{bc}$ | $4.89 \pm 1.08^{ab}$ |
| 404 | $8.26 \pm 1.09^{ab}$ | $5.28 \pm 0.65^{a}$ |
| 437 | $6.41 \pm 0.47^{bc}$ | $3.53 \pm 0.21^{c}$ |
| 449 | $6.02 \pm 0.88^{d}$ | $3.45 \pm 1.02^{c}$ |
| 482 | $6.93 \pm 0.72^{bc}$ | $4.42 \pm 0.38^{bc}$ |
| 1102 | $6.18 \pm 0.37^{cd}$ | $4.86 \pm 0.25^{b}$ |

[1] mg gallic acid equivalent/g DW.
[2] mg rutin equivalent/g DW. Means with different letters within column are significantly different; Values are means of three replications (±SD).

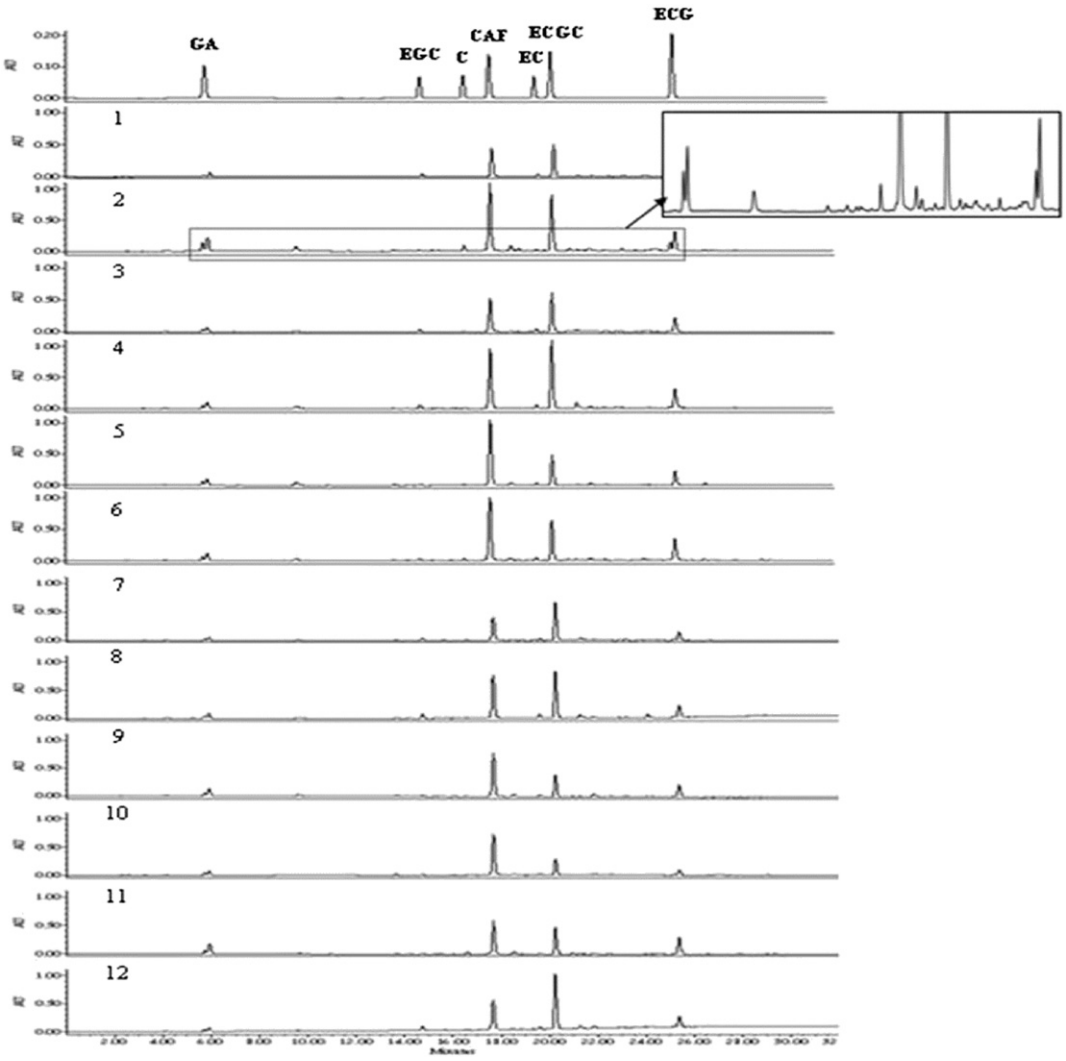


**Fig. 2.** HPLC chromatograms (280 nm) of reference standards in comparison with 12 *C. sinensis* samples. GA: gallic acid; EGC: (−)-epigallocatechin; C: (+)-catechin; CAF: caffeine; EC: (−)-epicatechin; EGCG: (−)-epigallocatechin-gallate; ECG: (−)-epicatechingallate; 1: sample Fashalam; 2: sample clone Iran 100; 3: sample 102; 4: sample 178; 5: sample 218; 6: sample 219; 7: sample 223; 8: sample 404; 9: sample 437; 10: sample 449; 11: sample 482; 12: sample 1102.

its antioxidative properties, epidemiological evidence shows that population with high consumption of green tea catechins demonstrate beneficial effect on the management of body weight, control of glucose level and risk factors related to cardiovascular diseases and cancer [25,26]. At least ten catechins have been reported in *C. sinensis* [15] and among these catechins, the most abundant are EGCG and ECG that are reported to be the primary component responsible for many pharmacological effects including antioxidant activity [26].

**Table 2**
Quantitative results of some important constituents analyzed in 12 tea clones.

| Clone | Contents (mean ± SD, mg/g) | | |
|---|---|---|---|
| | EGCG | ECG | CAF |
| Fashalam | 2.40 ± 0.08 | 0.68 ± 0.05 | 1.58 ± 0.09 |
| 100 | 3.80 ± 0.11 | 1.55 ± 0.14 | 3.78 ± 0.14 |
| 102 | 2.90 ± 0.17 | 1.12 ± 0.06 | 1.92 ± 0.13 |
| 178 | 4.90 ± 0.19 | 1.45 ± 0.06 | 3.54 ± 0.16 |
| 218 | 2.33 ± 0.13 | 1.14 ± 0.05 | 3.65 ± 0.18 |
| 219 | 3.00 ± 0.09 | 1.54 ± 0.06 | 3.55 ± 0.20 |
| 223 | 3.01 ± 0.16 | 0.80 ± 0.06 | 1.52 ± 0.18 |
| 404 | 3.56 ± 0.14 | 1.12 ± 0.09 | 2.69 ± 0.08 |
| 437 | 1.97 ± 0.14 | 1.01 ± 0.08 | 2.70 ± 0.24 |
| 449 | 1.60 ± 0.18 | 0.59 ± 0.07 | 2.43 ± 0.16 |
| 482 | 2.32 ± 0.12 | 1.38 ± 0.04 | 2.22 ± 0.18 |
| 1102 | 4.06 ± 0.20 | 0.90 ± 0.05 | 1.87 ± 0.23 |

**Table 3**
DPPH scavenging activities in the leaves of 12 clones of tea at the concentration of 250 μg/mL.

| Clone | Inhibition (%) |
|---|---|
| Fashalam | $56.29^{d}$ |
| 100 | $65.36^{a}$ |
| 102 | $51.72^{fg}$ |
| 178 | $61.83^{b}$ |
| 218 | $59.87^{c}$ |
| 219 | $57.71^{d}$ |
| 223 | $54.03^{e}$ |
| 404 | $55.04^{e}$ |
| 437 | $51.31^{g}$ |
| 449 | $53.28^{ef}$ |
| 482 | $22.67^{i}$ |
| 1102 | $49.59^{h}$ |

All analyses were mean of triplicate measurements. Results expressed in percent of free radical inhibition. Means not sharing a common letter were significantly different at $P \leq 0.05$.

**Table 4**
DPPH scavenging activities in the leaves of 12 clones of tea. BHT and α-tocopherol were used as controls.

| Clone | $IC_{50}$ (μg/mL) |
|---|---|
| Fashalam | 227.55[f] |
| 100 | 218.25[i] |
| 102 | 246.03[b] |
| 178 | 222.00[h] |
| 218 | 223.01[gh] |
| 219 | 228.52[ef] |
| 223 | 236.62[d] |
| 404 | 225.89[fg] |
| 437 | 241.49[c] |
| 449 | 231.18[e] |
| 482 | 234.44[d] |
| 1102 | 261.26[a] |
| BHT | 89.77 |
| α-Tocopherol | 60.39 |

All analyses were mean of triplicate measurements. Means not sharing a common letter were significantly different at $P \leq 0.05$.

**Table 5**
Pearson's correlation coefficients of DPPH, EGCG, ECG, total flavonoid, and total phenolic content.

| | Total phenolic | Total flavonoid | EGCG | ECG | Caffeine |
|---|---|---|---|---|---|
| DPPH | .85** | .88** | .84** | .83** | .59* |

Note: * represents moderate positive correlation; ** represents high positive correlation.

Results obtained by correlation analysis as depicted in Table 5 indicate that antioxidant activity had a positive correlation with the contents of total phenolics, total flavonoids, EGCG, ECG and CAF ($0.59 \leq r \leq 0.97$, $P < 0.05$). The highest correlation was observed between total flavonoid content and antioxidant activity ($r = 0.88$, $P < 0.01$), while the lowest correlation was found between antioxidant activity and CAF. Positive correlation between these five contents was also found in other elite tea varieties [27,28]. Unlike catechins, CAF has been given appalling reputation as it exhibits increased risks of coronary heart disease and produces various adverse effects such as palpitations, gastrointestinal problems, tremor, anxiety and insomnia particularly to sensitive people and this has promoted the production of decaffeinated tea. Until recently, CAF in tea is believed to work differently than in other beverages such as coffee due to the presence of L-theanine, a non-proteinic amino acid. A recent study shows that L-theanine has a propensity to antagonize the caffeine-induced rise in blood pressure [29] and it posses relaxing effect and can serve as anti-stress agent [30]. Furthermore, León-Carmona and Galano [17] found that CAF is excellent scavenger against hydroxyl radical (OH).

## 4. Conclusion

The 12 clones selected in the studies were those that have been proven to have a higher yield. However, the high yielding characteristics need to be combined with quality of the phytochemicals present. This study concludes that among all the tea clones studied, Iran 100 was found to be superior because of higher phytochemical contents such as flavonoid and phenolic compounds. Also, the highest antioxidant activity, total phenolics, total flavonoid, ECG and CAF content were observed in the clone, Iran 100. Therefore, it can be concluded that the clone, Iran 100 could be a better choice for establishing commercial farm for tea production.

## Conflict of interest

The authors have no conflicts of interest to declare.

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