Electronic Journal of Biotechnology 18 (2015) 433–438

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Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research Article

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

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ARTICLE INFO

*Article history:*
Received 18 May 2015
Accepted 26 August 2015
Available online 28 October 2015

*Keywords:*
*Camellia sinensis*
DPPH assay
Methanolic extract
Total flavonoid content
Total phenolic content

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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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2015.08.007

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.