Comparative Assessment Of Catechin And Gallic Acid Content In Different Brands Of Black And Green Tea

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Abstract. Levels of catechins, gallic acid in a total of 14 samples of different tea samples commercialized in Iraq have been evaluated via High Performance Liquid Chromatography using Photodiode Array Detector. Large variations in the two compounds concentrations were observed and detected. The levels ranged from 96 to 176 µg L⁻¹ and 13 to 88 µg L⁻¹ for catechin and gallic acid concentration. The obtained results showed that green tea has a higher level of content of catechin than black tea. The process of fermentation during black tea production minimize the level of catechin concentration significantly. This study will be useful for the assessment of antioxidant component in various tea samples, and it will also be of interest for people who like drinking this beverage.

Keywords: Green tea, catechin, gallic acid, HPLC, Black tea

1. Introduction

Tea which is an aqueous solution of leaf of plant from Camellia family. Tea consider to be the largest consumed drink in the earth, also it is considered to be an important source of antioxidants and other nutrition. In general, there are three types of teas; green, Chinese and black tea. These types are different in methods of production, preparation and drinking in around the world1-6.

Tea has several chemicals include; phenols, polyphenols, caffeine, amino acids, carbs., elements etc. It is well known to be a medicine, in recent researches, tea show high activity as antioxidant, the published articles show that consuming more tea will lead to minimize the possibility to have cancer, such as stomach, oral cavity and lung cancer. Many reports have been confirmed that catechin and gallic acid are the most important compounds among the other tea constituents6-10.

It is known that the in-vitro antioxidant properties of tea polyphenolic led to the potential health benefits of tea consumption. Numerous epidemiologic studies claimed relationships between consumption of tea and incidence of cardiovascular disease. The mechanism of action of tea on human health not only
as an antioxidant action, (such as the LDL), but also consider to be as anti-inflammatory, and thermogenesis\textsuperscript{11-16}.

The antioxidant behavior in different types of tea is determined via common analytical methods, such as oxygen radical absorption capacity (ORAC) and ferric reducing ability of plasma (FRAP). The basic principle of ORAC is measuring the fluorescence when it is exposed to the stable radicals and the antioxidants. While FRAP is based on the recovery of Iron (III) complex -tripyrindyltriazine with Iron (II) at low pH\textsuperscript{16,17}.

In this article, we quantitatively measured the concentration of catechine and gallic acid in tea samples, the determination has been done via HPLC-UV method and the results obtained are extremely valuable for food composition database.

2. Materials and methods

2.1. Samples

Tea leaves samples (14 samples) have been collected and purchased from the local market in Diwaniah city-Iraq, these samples were locally produced or imported from different countries.

2.2. Instrumentation

2.3. UV-absorption spectrophotometer

UV absorption spectra were obtained by using a double-beam spectrophotometer type UV1650 from Japan, the instrument use a xenon type lamp, 2nm fixed during the analysis band-pass. The solutions were measured using 4 mL quartz type cuvette with a 1cm cuvette path length absorption.

2.4. Chromatographic detection of EGCG and gallic acid

HPLC sample analysis was performed using HPLC system which consisted of a system controller model SCL10VP. The HPLC system equipped with gradient pump model LC10AVP, an (UV) detector model S-P-D10-AVP, and a non-line de-gasser model DG-URA.

2.5 Reagents

To remove any possibility of contamination, all glassware was cleaned and washed with distilled water before and after use, then immersed in a 7M HNO\textsubscript{3} solution for 12 hours before further use, then washed again with distilled water several times. Gallic acid was purchased from Merck and catechin was from Sigma-aldreich. All other solvents were HPLC grade.

2.6 Preparation of standard solution

Stock solutions of EGCG and gallic acid (150 μg mL\textsuperscript{-1}) have been prepared via dissolving 1.5 mg of standards solutions in 10mL of methanol. The stock solutions were kept in black container at 5 °C prior to use, stock solutions were examined using Uv spectroscopy to be sure no possible degradation by light for the solutions, and they used within period of 6 months of preparation. Working solutions of EGCG and gallic acid prepared each day by serial dilution of stock solution.
2.7. Sample preparation for antioxidant determination

100 g Tea leaves samples were crushed, powdered, and extracted for 4 times with 25 mL of 80 percent MeOH for 1 h and then 3 times with 25 mL of 80 percent MeOH containing 0.20 % HCl for 1 h. The extracts were collected and added to each, then filtered. The solution was filtered for second time through 0.45 µm of nylon membrane filter (Millipore). The process was repeated for 3 time and a total volume obtained was evaporated to dryness then re-dissolved in 4 ml methanol.

3. Results and discussion

3.1. HPLC analysis

For HPLC separation, a generally used C<sub>18</sub> HPLC separation column has been selected as a reverse phase stationary phase column, it has length equal to 10 cm, and a radius of 2 mm the average particle size was 5 µm. the C<sub>18</sub> column which is used previously to perform the analysis following work that published previously 18-19. The chromatographic separation was done using a 80 percent acetonitrile as a mobile phase, a flow rate of 1.5 ml/min and a constant column temperature at room temperature, figure[1] shows a typical HPLC chromatogram for injection of 20 ppb standard solution of catechin and gallic acid in methanol, using 282 nm as absorption wavelength inside the UV detector.

In this article we use 3 chromatographic HPLC runs of the standard solution obtained from 3 different injections of 20 µL in volume which provided 9.8 ± 0.2 and 29.5 ± 0.5 minutes retention time for catechin and gallic acid respectively. To generate a calibration curve, 5 different concentrations of both catechins and gallic acid were made using distilled water and injected directly into HPLC.

Figure 1 Typical HPLC chromatogram for standard mixture solution of catechin and gallic acid
3.2. Determination of Catechin and gallic acid via HPLC:

Commercial samples have been collected from local market in Al-Diwaniah city, Iraq, these samples were either imported or locally produced. These samples were prepared for analysis and injected to HPLC-Uv detector. Figure [2] show an example of injection of methanolic extract of tea.

![Figure 2 HPLC chromatogram for injection of sample extract](image)

The concentrations of catechin and gallic acid in the tea samples that obtained from HPLC are presented in Table1. The results show a variation in both compounds concentrations and there is no significant difference in catechin and gallic acids in all black tea samples among the different brands, a significant difference was found in catechin concentration between black and green tea as shown in figure [3], the green tea show a higher content of catechin.

![Figure 3 Catechin content comparison in tea samples](image)
The samples of green tea showed higher concentrations of catechin, about 35% more, when compared to black tea samples. The significant difference obtained in catechin concentration between the black and green tea was in agreement with the results reported previously\textsuperscript{20}, the difference is due to the variation in the degree of fermentation during the process of production. The Black leaves tea was produced by a after harvest fermentation, the autoxidation process has been catalyzed via polyphenol oxidase enzyme, while a steaming process has been done for the green tea leaves to inactivate the oxidase enzyme prior to drying. The analyzed sample showed the catechin (which is the major constituent in tea leaves\textsuperscript{20}) concentration level ranged from 96-176 µg L\textsuperscript{-1}. The high level of it as antioxidant is well described. Catechin identified as inhibitor for several types of cancer. The gallic acid content varied between 13 to 88 µg L\textsuperscript{-1}. Figure [3] show a comparison between the concentration of catechin and gallic acid which shows clearly the higher concentration values of catechin compared to ones obtained from gallic acid.

| sample | Brand                  | Catechin conc. µg L\textsuperscript{-1} | Gallic acid conc. mg L\textsuperscript{-1} |
|--------|------------------------|----------------------------------------|----------------------------------------|
| 1      | Ahmed                  | 102 ± 10                               | 13 ± 1.4                               |
| 2      | Ahmed with haail       | 112 ± 11                               | 17 ± 1.3                               |
| 3      | Wazza                  | 101 ± 9                                | 85 ± 2.9                               |
| 4      | Kafeel                 | 96 ± 11                                | 81 ± 3.1                               |
| 5      | Gazalain               | 98 ± 12                                | 43 ± 4.1                               |
| 6      | Local 1                | 100 ± 9                                | 88 ± 3.9                               |
| 7      | Local 2                | 107 ± 8                                | 25 ± 4.1                               |
| 8      | Local 3                | 108 ± 18                               | 63 ± 2.1                               |
| 9      | Local 4                | 101 ± 21                               | 74 ± 3.8                               |
| 10     | lipton                 | 99 ± 8                                 | 49 ± 3.0                               |
| 11     | English breakfast tea  | 98 ± 7                                 | 62 ± 3.2                               |
| 12     | Lipton green tea       | 146 ± 12                               | 67 ± 2.0                               |
| 13     | BigElow green tea      | 176 ± 12                               | 51 ± 1.8                               |
| 14     | Rabee express green tea| 159 ± 19                               | 45 ± 1.7                               |

Figure 4 comparison between catechin and gallic acid content in tea samples
In general, there is a variation in the concentration of both compounds in different type of tea and this variation is due to, weather, season, age of the tea leaves and soil condition.

4. CONCLUSION

In conclusion, the levels of catechin and gallic acid in the studied tea samples were comparable values with the results obtained from other research articles.; however, the results show a wide ranged values. Tea is an significant dietary source of different compounds mainly catechin and gallic acid, which help to reduce incidence of malignancy. The tea catechin and gallic acid content, present in high level, the content of these compounds is correlated to the feature of tea leaves and to the level of fermentation during tea production. In conclusion, tea could be an significant dietary source of different polyphenols with antioxidant activity, and future studies required to be designed to precisely assess their presence and bioavailability.

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