

## COMPARATIVE STUDY BETWEEN THE EGYPTIAN AND CHINESE GREEN TEA (CHEMICAL COMPOSITION, PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY)

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**ABSTRACT:** *Chemical composition and the antioxidant activity of Egyptian and Chinese tea extracts (ethanol, water, hot water and boiling water) were investigated.*

*Egyptian green tea leaves contains total ash 8.77%, total lipids 7.1%, crude protein 17.6%, and total carbohydrates 7.5%. While Chinese green tea leaves contains total ash 7.8%, total lipids 7.05%, crude protein 18.2%, and total carbohydrates 7.44%. Total phenolics in Egyptian green tea extracts has been ranged from 113.7 to 259.1 mg/g while total flavonoids has been ranged from 87.6 to 185.9 mg/g, comparing with 89.4 to 229.1 mg/g and 79.7 to 181 mg/g of total phenolic and total flavonoid respectively in Chinese green tea extracts. HPLC results showed that Egyptian and Chinese green tea were found to contain 18 of phenolic compounds, among them Caffeine, epicatechin, cinnamic acid, reverstrol, Salycilic acid and Ferulic acid were the major active constituents. All extracts showed high antioxidant activity in both concentration (2.5 and 5%). Ethanolic extract showed the highest activity in reducing power assay compared other extracts and boiling water extract showed the lowest activity. Egyptian green tea showed high content from both total phenolics and falvonoids and also showed high antioxidant activity compared with Chinese green tea.*

**Key words:** *Green tea – Total phenolics – Antioxidant activity – Reducing power.*

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### INTRODUCTION

Tea (*Camellia sinensis*) is the most popular beverage consumed by human society worldwide, next only to water. Every year, about 2.5 million tons of teas are produced and consumed in the world (Han *et al.* 2005). Its leaves are dark green and shiny, opposite and round, Its flowers are large, white, pink or red while its fruits are small and brown. (Chu and Juneja, 1997 and Balentine *et al.*, 1997). Tea contains a wide assortment of bioactive constituents, most of which are found in two groups, alkaloids and polyphenols; alkaloids found in tea include caffeine, theobromine, and theophylline. (Tyler . *et al.* , 1998). There are three main varieties of tea: green, black, and oolong, and all of them are derived from the

leaves of the *Camellia sinensis* plant. As Green tea is made from unfermented leaves it contains the highest concentration of powerful antioxidants called polyphenols. (Narotzki *et al.*, 2012 and Araghizadeh *et al.*, 2013). Green tea leaves contain flavonoids as well as phenolic acids which can make up to 30% dry weight of fresh leaves and only 10 % of dry weight of black tea (Wang *et al.*, 2000). Green tea polyphenols show an antioxidant activity *in vitro* by scavenging reactive oxygen and nitrogen species and act as chelating agents for redox-active transition metal ions; i.e. green tea polyphenols can chelate metal ions like iron and copper to prevent their participation in Fenton and Haber-Weiss reactions (Singh *et al.*, 2002). (Hajimahmoodi

*et al.*, 2008) found that, a flavonoid and antocyanidin content of the tested green tea samples possess relatively high antioxidant activity due to contribution of phenolic compounds. The aims of the present study were to determine the chemical composition, total phenolic compounds of Egyptian and Chinese green tea and to qualify the antioxidant activity of different extracts prepared from both tea types.

## **MATERIALS AND METHODS**

### **1- Plant collection and Identification.**

Leaves of Egyptian Green Tea (*Camellia sinensis*) were collected from Talat, Senoris, Faiom, Egypt in September 2013 and Leaves of Chinese Green Tea (*Camellia sinensis*) were collected from local Egyptian Market. Leaves were identified in Horticulture department, Faculty of Agriculture, Minufiya University.

Plant samples were washed and air-dried for 24 hours, then dried at 50 °C for 24 hours; the dried leaves were grinded into fine powder.

### **2- Determination of chemical composition in green tea.**

Total nitrogen was determined (dry basis) according to the modified micro-Kjeldahl (Pirjo and Pekka 1996) method as described by the Association of Official Analytical Chemists, A. O. A. C., (1990). The crude protein contents were calculated using the conversion factor 6.25. Crude lipid was determined according to A. O. A. C., (1990). The sugars were determined according to the method of Dubois *et al.*, (1956). Ash content was determined by ignition of dried sample at 550°C until a constant weight according to (A.O.A.C, 1990).

### **3- Extraction of phenolic compounds.**

Extraction of phenolic compounds was conducted according to the method described by (Gulcin *et al.*, 2002) as follows:

A known weight of (2 gm) of dry samples was macerated in 10-15 ml from (water, ethanol 80 %, hot water and boiling water). At the end, clarified extract was completed to 100 ml. using the same solvent.

### **4- Determination of free phenolic compounds content.**

The concentration of free phenolic compounds in each extract was determined colorimetrically by the method of Folin-Ciocalteu's as described by (Gulcin *et al.*, 2002) 5 ml of each extract was diluted to a total volume 25 ml. with distilled water, and 1 ml of the solution extract was pipetted into a flask. Then 46 ml of distilled water and 1 ml of Folin-Ciocalteu's reagent was added and mixed thoroughly. The mixture was left to stand for 3 min to which 3 ml of 20% sodium carbonate solution was then added. After 120 min of incubation at ambient temperature with constant shaking, the resulting absorbance was measured at 760 nm against reagent blank. Measurements were carried out in duplicate and a calibration curve was formed using gallic acid (GA). The results were expressed as g GAE/100g dry matter.

### **5- Determination of total flavonoids contents.**

The total flavonoids contents were determined using the method reported by (Dewanto *et al.*, 2002). Briefly, an aliquot (250 µL) of each extract or a standard solution was mixed with 1.25 mL of deionised water followed by 75 µL of a 5% NaNO<sub>2</sub> solution. After 6 min, 150 µL of a 10% AlCl<sub>3</sub> · 6H<sub>2</sub>O solution was added to each mixture. After 5 min, 0.5 mL of 1 M NaOH was added, and the total volume was adjusted to 3.0 mL with deionised water. (+)-Catechin was used as a standard. The

absorbance at 510 nm, which was corrected using a blank, was then determined and the results were expressed as mg of (+)-catechin equivalents (CE)/100 g flavonoids weigh.

## **6- HPLC separation, identification and quantification of phenolic compounds.**

A modified method of (Zuo *et al.* 2002) was used. A Shimadzu LC 20 AT HPLC fitted with a SIL 20A auto sampler and a SPD-20 UV Visible detector with a class LC 10 chromatography workstation was used for the analysis of the prepared samples. A Luna TM 5  $\mu$ M C18, 25 cm x 4.6 mm i.d (Phenomenex, Torrance, CA, USA) column with a Reodyne precolumn filter 7335 model was used. All solvents were filtered through a 0.45  $\mu$ m millipore membrane filter disk and degassed before injection into a HPLC system. A gradient elution was carried out using the following solvent systems: Mobile phase A (acetonitrile / acetic acid/double distilled water- 9/2/89 v/v/v), Mobile phase B (acetonitrile/acetic acid/double distilled water - 80/2/18 v/v/v). The mobile phase composition for a binary gradient condition was started at 100% solvent A for 10 min then over 15 minutes a linear gradient to 60% mobile phase A, 32% mobile phase B and held at this composition for 10 min. The condition was reset to 100 % mobile phase A and allowed to equilibrate for 10 min before the next injection. The flow rate of the mobile phase was 1 ml/ min and the temperature at the column was performed at  $35 \pm 0.5$  °C. The identification of individual catechins was carried out by comparing the retention times and UV- absorbance of unknown peaks with peaks obtained from the mixed known standards under the same conditions. The quantification of catechins was performed at 278 nm and was achieved using a caffeine external standard with a calibration curve  $R^2 = 0.9984$  in conjunction

with the consensus individual catechins relative response factor (RRF) values with respect to caffeine calculated on dry matter basis. Total catechins as percentage by mass on a sample dry matter basis was given on the summation of individual catechins.

## **7- In vitro antioxidant activity reducing Power assay.**

A spectrophotometric method (Oyaizu 1986) was used for the measurement of reducing power. For this determination 2.5 ml of each of the extracts were mixed with 2.5 ml of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After adding 2.5 ml of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionised water and 1 ml of 0.1% of ferric chloride, and the absorbance was then measured at 700 nm: higher absorbance indicates higher reducing power. Vit.C was used as standard.

## **RESULTS AND DISCUSSION**

### **1- Chemical composition of Egyptian and Chinese green tea leaves:**

The obtained results in Table (1) indicate that Egyptian green tea leaves contains total ash 8.77%, total lipids 7.1%, crude protein 17.6%, and total carbohydrates 7.5%. While Chinese green tea leaves contains total ash 7.8%, total lipids 7.05%, crude protein 18.2%, and total carbohydrates 7.44%.

The result are in accordance with those of Shu *et al.*, (2003) and Yung *et al.*, (2003), they refer that green tea chemical composition is complex: proteins 15 - 20% dry weight, carbohydrates 5 - 7% dry weight, minerals and trace elements 5% dry weight

## 2- Total phenolic compounds and total flavonoids of different green tea extracts.

Data in Table (2) showed that high total phenolics and total flavonoids contents for most of Egyptian green tea extracts are higher than that in Chinese green tea extracts.

Total phenolics in Egyptian green tea extracts has been ranged from 113.7 to 259.1 mg/g while total flavonoids has been ranged from 87.6 to 185.9 mg/g, comparing with 89.4 to 229.1 mg/g and 79.7 to 181 mg/g of total phenolic and total flavonoids respectively in Chinese green tea extracts .

These data agree with those of Wang *et al.*, (2000) who found that green tea contain 30% of total phenolic compounds, Lee *et al.*, (2014) who indicated that total catechins are ranged between 200.4 to 215.9 mg/g green tea in different green tea types in Korea, and Balentine *et al.*, (1992) and Kong (1993) who Showed that green tea contains 30-42% catechins of total dry weight, while black tea contains 3-10% and oolong 8-20%. Graham (1992) showed also that almost 80% of the tea consumed throughout the world each year is black; less than 2% is Oolong, and 20% green tea.

**Table (1): Chemical composition of Egyptian and Chinese green tea leaves.**

Chemical composition	Egyptian green tea leaves (g/100g)	Chinese green tea leaves (g/100g)
Ash	8.77	7.8
Total lipids	7.1	7.05
Crude protein	17.6	18.2
Total carbohydrates	7.5	7.44

**Table (2): Total phenolic compounds and total flavonoids content of Egyptian and Chinese green tea extracts.**

	Egyptian green tea		Chimes green tea	
	Total phenolics mg/g	Total flavonoids mg/g	Total phenolics mg/g	Total flavonoids mg/g
Ethanollic extract	259.1	185.9	229.1	181
Cold water extract	221.2	165.2	212.7	168.4
Hot water extract	169.9	121	157.4	111.5
Boiling water extract 5 min	113.7	87.6	89.4	79.7

**3- Quantitative analysis of phenolic compounds in Egyptian and Chinese green tea leaves.**

Phenolic compounds in Egyptian and Chinese green tea leaves were analyzed by High Performance Liquid Chromatography (HPLC), and concentration of all tested phenolic compounds are given in Table (3). From this table it was found that Egyptian green tea leaves contains caffeine as the main phenolic compound which reached 28.6 mg/g, followed by epicatechin 13.2 mg/g then catechin 5.7 mg/g. Analysis of Chinese green tea leaves showed that caffeine reached 27.74 mg/g, followed by epicatechin 12.97 mg/g, then catechin 6.22

mg/g. Results of phenolic compounds analysis are nearly similar to those reported by (Lee *et al.*, 2014) who studied the main phenolic compounds in different green tea types in Korea, and found that green tea contains caffeine 26.74 to 29.54 mg/g and epicatechin 11.84 to 12.59 mg/g. These results agree also with that of Graham (1992) who found that caffeine accounted 3.5% of dry weight in green tea leaves. Most of the polyphenols in green tea are flavanols, commonly known as catechins e.g. (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (Ahmad *et al.*, 2000 and Tsuchiya 1999).

**Table (3): Phenolic compounds in Egyptian and Chinese Green tea**

Phenolic compounds	Content (mg/g )	
	Egyptian green tea	Chinese green tea
Catechein	5.7	6.22
Epicatechein	13.2	12.97
Caffeine	28.6	27.74
Caffeic acid	2.39	3.6
Ellagic acid	1.56	2.23
Cinnamic acid	8.75	3.06
Reverstrol	6.79	1.18
Salycilic acid	6.64	5.42
Pyrogallol	3.47	3.22
P-Coumaric acid	3.5	1.46
Iso-Ferulic acid	3.49	2.98
Benzoic acid	2.82	1.67
Chlorogenic acid	2.76	1.53
Coumarin	2.82	1.17
Catechol	2.73	8.18
Alpha-Coumaric	1.82	2.34
Ferulic acid	1.12	5.37
Gallic acid	1.09	2.01

#### **4- Reducing power activity for different green tea extracts**

Tea, particularly green tea, is a potentially rich dietary source of antioxidant power. Various studies *in vitro* have demonstrated radical trapping antioxidant properties in black and green tea extracts and of individual polyphenolic compounds found in tea (Rice-Evans *et al.*, 1996; Zhang and Shen, 1997).

Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important mechanism in phenolic antioxidant action (Nabavi *et al.*, 2009). In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of  $Fe^{+3}$  to  $Fe^{+2}$  by donating an electron.

Data in Figure (1) showed reducing power assay results for different Egyptian and Chinese green tea extracts (2.5% concentration). For Egyptian green tea: ethanolic extract it was (36.14 mMol Ascorbic Eq) followed by cold water extract (19.86 mMol Ascorbic Eq) hot water extract (17.86 mMol Ascorbic Eq.) and boiling water extract (13 mMol Ascorbic Eq); while it was for Chinese green tea ethanolic extract (35.28 mMol Ascorbic Eq) followed by cold water extract (21.86 mMol Ascorbic Eq) hot water extract (11.87 mMol Ascorbic Eq) and boiling water extract (8.28 mMol Ascorbic Eq).

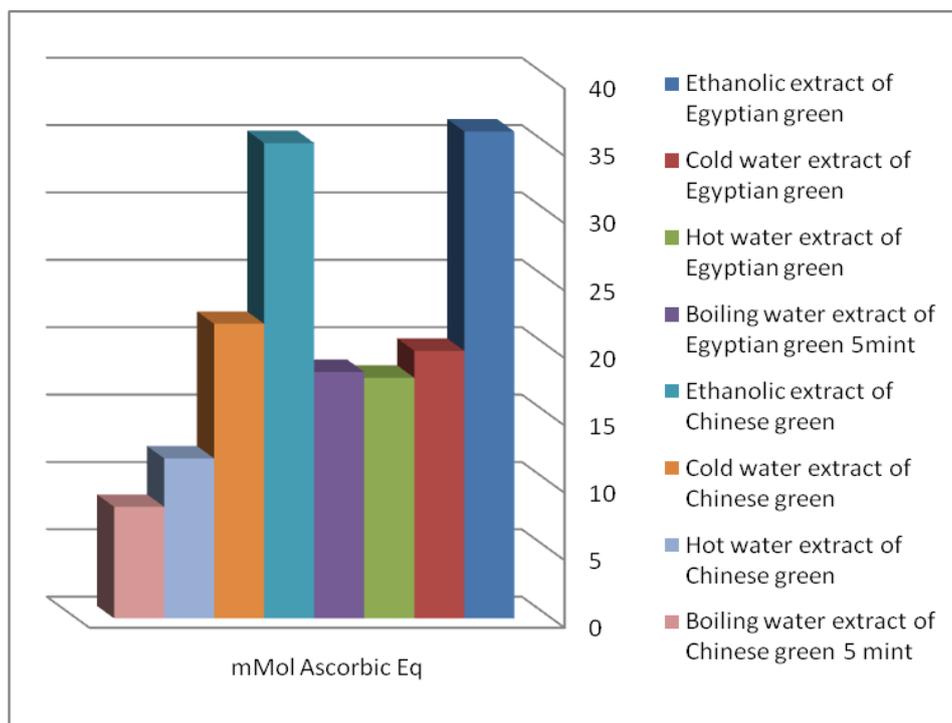
Data in Figure (2) showed reducing power assay results for the previous

different tea extracts (5% concentration); and it was for Egyptian green tea ethanolic extract (644 mMol Ascorbic Eq) followed by cold water extract (501 mMol Ascorbic Eq.) hot water extract (175 mMol Ascorbic Eq) and boiling water extract (149 mMol Ascorbic Eq.); while that of Chinese green tea ethanolic extract was ( 442 mMol Ascorbic Eq), followed by cold water extract (336 mMol Ascorbic Eq), hot water extract (145 mMol Ascorbic Eq.) and boiling water extract (119 mMol Ascorbic Eq.).

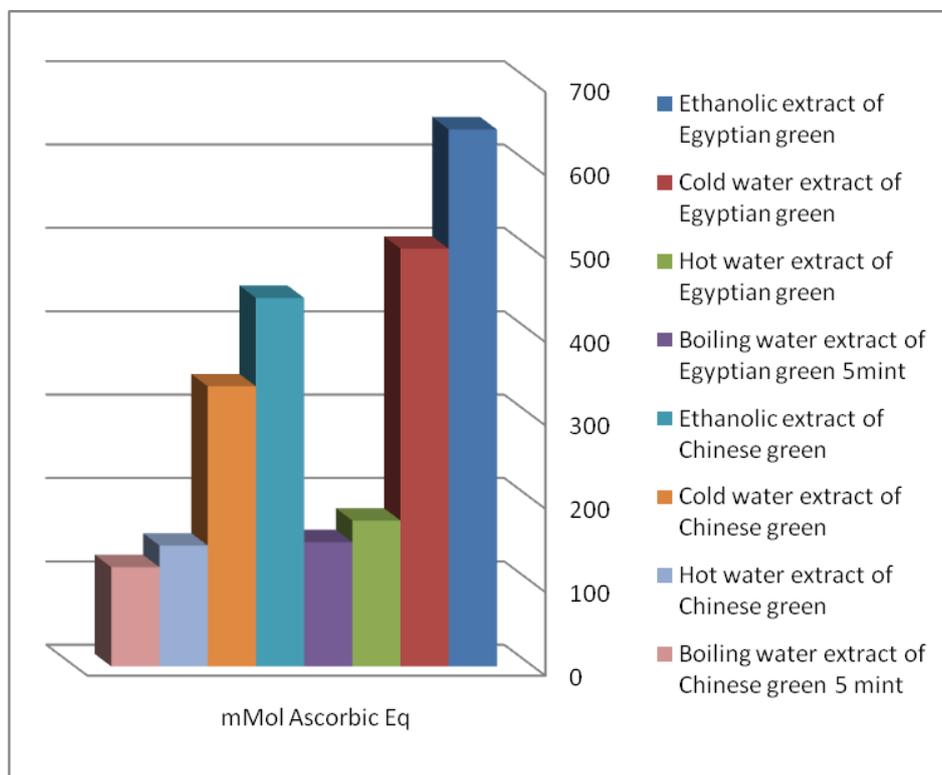
Our data are in line with that of Benzie and Szeto, (1999) who reported that one cup of tea of usual strength (1-2%), can provide the same potential for improving antioxidant status as around 150 mg of pure ascorbic acid (vitamin C). The antioxidant properties of green tea were the strongest among the various types of teas. It contains considerable amounts of catechins, which are effective in antioxidant properties. These phenolic compounds usually are the most abundant water-soluble components in the tea and responsible for its effective antioxidant properties (Balentine *et al.*, 1997).

Tea polyphenolics also have electron-donating antioxidant properties, the relative activity of the different polyphenolic compounds being related to the number and location of the hydroxyl groups on B and C rings and the presence of the galloyl moiety (Lin *et al.*, 1996; Miller *et al.*, 1996; Paganga *et al.*, 1996).

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**Fig. (1): Reducing power activity for different green tea extracts (2.5% concentration)**



**Fig. (2): Reducing power activity for different green tea extracts (5% concentration).**

## Conclusion

Both Egyptian and Chinese green tea were found to contain 18 of phenolic compounds, among them Caffeine, epicatechin, cinnamic acid, reverstrol, Salycilic acid and Ferulic acid were the major active constituents. The data showed that Egyptian green tea extracts were more effective than Chinese green tea as antioxidant and ethanol extract was the more effective one as antioxidant.

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## دراسة مقارنة بين الشاي الأخضر المصري والصيني (التركيب الكيميائي – المركبات الفينولية – النشاط المضاد للأكسدة)

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### الملخص العربي

تمت دراسة التركيب الكيميائي للشاي الأخضر المصري والصيني وكذا المركبات الفينولية والنشاط المضاد للأكسدة لمستخلصاتهم المختلفة.

ووجد أن الشاي الأخضر المصري يحتوي على ٨.٧٧ % رماد ، ٧.١ % دهون كلية ، ١٧.٦ % بروتين وأخيرا ٧.٠٥ % كربوهيدرات . في حين يحتوي الشاي الأخضر الصيني على ٧.٨ % رماد ، ٧.٠٥ % دهون كلية ، ١٨.٢ % بروتين وأخيرا ٧.٤٤ % كربوهيدرات . وتتراوح الفينولات الكلية في المستخلصات المختلفة للشاي الأخضر المصري بين ١١٣.٧ إلى ٢٥٩.١ مجم/جم في حين تتراوح الفلافونات الكلية بين ٨٧.٦ إلى ١٨٥.٩ مجم/جم مقارنة بمحتوي فينولات كلية في مستخلصات الشاي الأخضر الصيني تتراوح من ٨٩.٤ إلى ٢٢٩.١ مجم/جم في حين تتراوح الفلافونات الكلية لنفس المستخلصات من ٧٩.٧ إلى ١٨١ مجم/جم.

وأظهرت نتائج التحليل الكروماتوجرافي السائل عالي الأداء (HPLC) أن كلا النوعين يحتوي على ١٨ مركب فينولي كان أهمهم مركبات: الكافيين ، الإبيكاتشيين ، السينامبيك ، الريفسترول، حمض السالسليك و حمض الفيروليك. كما أظهرت كل المستخلصات المختبرة نشاط مضاد للأكسدة عالي بتركيزات (٢.٥ و ٥%). وقد أظهرت المستخلصات الكحولية (كحول الإيثانول) النشاط الأعلى كمضاد للأكسدة بطريقة القوة الإختزالية (Reducing power) في حين أظهرت المستخلصات التي تم فيها غلي الشاي النشاط الأقل. ويمكن القول إجمالاً بأن الشاي الأخضر المصري أظهر محتوى اعلي في الفينولات الكلية والفلافونات الكلية وكذا أيضا أظهر نشاطا أعلى كمضاد للأكسدة معمليا مقارنة بالشاي الأخضر الصيني.

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