TOTAL POLYPHENOLS, TOTAL CATECHINS CONTENT AND DPPH FREE RADICAL SCAVENGER ACTIVITY OF SEVERAL TYPES OF VIETNAM COMMERCIAL GREEN TEA

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ABSTRACT: *Thirty types of commercial Vietnamese-brand teas (21 green teas, 5 black teas, 4 oolong teas) in Ho Chi Minh city were investigated for total polyphenols content (TPC-acid gallic equivalent), total catechins content (TCC-catechin equivalent) and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenger activity (RSA - Vitamine C equivalent). It was found that green tea had the highest TPC (15.06 ± 0.53 ppm), TCC (7.33 ± 1.71 ppm) and RSA (288 ± 23 mg) in three grams dried tea, higher than those of oolong tea, whereas black tea had the lowest TPC, TCC, RSA. However, high inconsistency and high deviation from mean values were observed within green tea group. Unexpectedly, several green tea types had low TPC, TCC, and RAC, even as low as those of black tea. This evidence demonstrated the instability and unevenness of green tea quality in Vietnam market.*

Key words: *Green tea, DPPH radical scavenger activity, total polyphenols content, total catechins content.*

1. INTRODUCTION

Tea has been one of the most consumed beverages in all over the world. Tea is used not only as fresh drink but also as traditional herb which has many benefits for human health. Tea has been believed and recently proven in several scientific articles about its abilities, such as anticancer, anti tumor, anti-aged. Moreover, tea is able to help relieve blood pressure, reduce heart attack rate....[6, 11, 13, 14]. This is primary because of the affect of polyphenols, tea main components, which could be as high as 30 – 35% in dried weight of fresh tea shoots [2, 3]. Tea polyphenols consist of several types of catechins compounds (usually 5 or 6, in some tea breeds the number of catechins compounds are 8, 9 or more), including C, EC, EGC, ECG, EGCG, ECG....[2, 16]. These catechin compounds have showed strong antioxidant activity, especially in free radical scavenger acting (oxy singlet, triplet, hydroxyl, superhydroxyl...) and metal chelating (to prevent catalyst effect of metal ion in oxidative reactions) [10, 11, 13, 14, 15].

Three most common of tea products are green, oolong and black tea. This category bases on tea processing. In green tea, all catechin compounds are intentionally left intact, but part of these in oolong tea and almost all in black tea are oxidized to theaflavines, thearugubins,....[2, 12, 16]. These oxidized products are responsible for the yellow color of oolong tea brewing water and deep yellow, reddish color of black tea brewing water [2].

Because oxidized products of catechins compounds have antioxidant activity lower than that of catechins compounds [15], and because of the nature of tea processing, green tea has highest catechins content, therefore has stronger antioxidant activity than that of two other teas, and also provides more healthy benefits. As a result, total polyphenols and total catechins content in green tea are two important factors in evaluating the quality of green tea. In several

countries such as China, Japan, India..., which are the biggest tea exporter and have long traditional of cultivating, producing, consuming and exporting tea, TPC, TCC become standard factors, which have to be considered in scanning and investigating quality of green tea [13].

Vietnam, one of the big tea exporters in the world (tenth in 2004), has already had plan to expand tea planting area and the tea industry capacity [1]. But quality assurance in tea process is still inadequate and out of date. Therefore it is necessary to carry out surveys to determine TPP, TCT and RSC of available tea products in Vietnam market and establish a reference for future development of tea process quality management.

2. MATERIALS AND METHODS

Tea: green tea (21 types), oolong tea (3 types), black tea (5 types), all are Vietnamese brands and were purchased at several markets and supermarkets in Ho Chi Minh City.

(-) Catechin (HPLC 99%) from Sigma Aldrich; Folin Ciocalteu agent, Vanillin from Mersk; other chemicals and solvents are analytical purity grade.

Brewing method : based on instructions printed on label of products, 3g of dried tea (5 – 7% humility) was brewed in 100ml distilled water at 100°C in 5 minutes, after that, cooled down to room temperature and filter to get brewing water.

This procedure was repeated several times until water did not change color when adding drops of indicator solution $FeCl_3$ 3% / HCl 0.1 (dark blue color appears if there are polyphenols in water) to extract all polyphenols in tea products. All extractees are accumulated to determine TPP, TCT, and RSC later.

Color of brewing water: brewing waters was measured by Minolta CR-300 colorimeter, with Lab color space system. (L: light – dark factor, a: green – red factor, b : blue – yellow factor).

Total polyphenols determination: Folin ciocalteu method [6, 12], is one of several methods which are used to determine total polyphenols. Polyphenols reduce Folin agent (yellow solution of polyphospattungstenate and molydate) in mild base medium to form deep blue color. The procedure was modified from [5], as follow:

- 0.5 ml of sample (distilled water is used for blank) was added to 10 ml flask, and diluted with 4.5 ml distilled water. Next, 0.2 ml Folin agent, 0.5 ml saturated Na_2CO_3 solution were added, respectively. The solution was shaken, added water to 10 ml and left for color development in 1 hour. After that, solution was measured at 725nm in Jenway 6505 UV-VIS spectrophotometer.

- Total polyphenols content was calculated from absorption value and linear regress equation using acid gallic as standard. Results was shown as ppm GE (Gallic acid Equivalent)

Total catechins determination: Vanillin-HCl method was used to determine total catechin compounds [8]. Vanillin high selectively reacts to catechin and leucoanthocyanidin compounds at 6, 8 positions, solution changes from colorless to pink when add concentrated HCl.

- 0.1ml of sample (methanol for blank) was diluted with 0.9ml methanol, after that 2.5ml of vanillin solution (1% in methanol) was added, followed by 2.5 ml of HCl 9N (diluted from concentrated HCl with methanol). The solution was left for color development in 30 minutes and measured at 500nm in UV-VIS spectrometer.

- Total catechin content was calculated from absorption value and linear regress equation using (+)- catechin as standard. Results was shown as ppm CE (Catechin equivalent)

DPPH radical scavenger activity determination: In the procedure, DPPH free radical (violet color) was reduced by antioxidant. The stronger antioxidant, the fader solution color is. The free radical scavenger activity was calculated from EC50, which is the concentration of antioxidant in solution that reduces the absorption half of its begin value. The procedure was described in [3, 9], and modified as follow:

- 0.5 ml of known concentration sample (methanol for blank) was diluted with 3.5 ml methanol. 0.5 ml of DPPH solution (1% in methanol) was added, final solution was shaken and left stand in dark for 30 minutes, then measured at 517nm in UV-VIS spectrometer.
- EC50 was calculated and compared with EC50 of acid ascorbic (Vitamin C).

3. RESULTS AND DISCUSSIONS

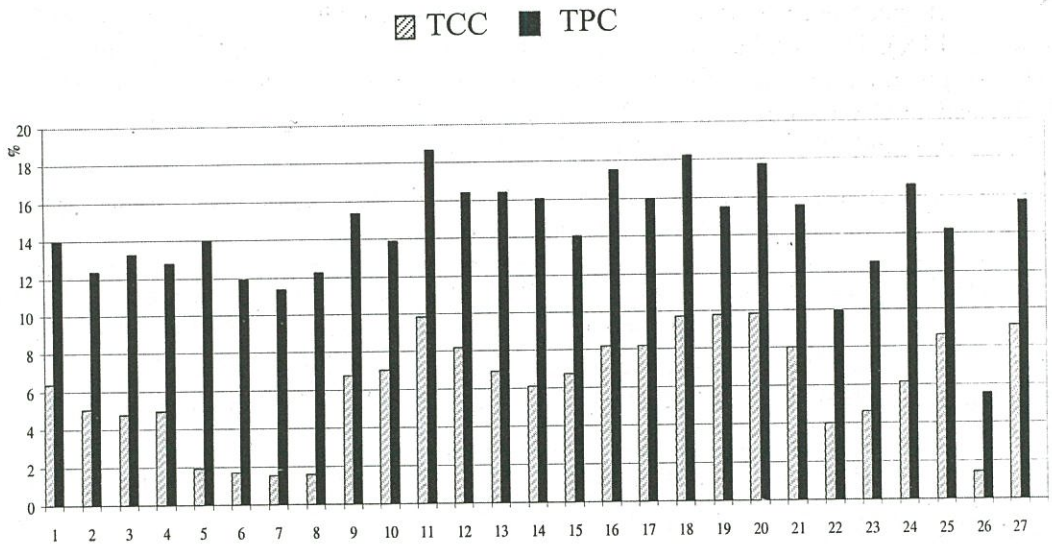
3.1. Color of tea brewing water

Table 3.1. Color of tea brewing color

Tea types	Color
Oolong tea	Greenish yellow – yellow – reddish yellow
Black tea	Deep yellow – reddish yellow – dark yellow
Green tea	Greenish yellow – yellow

Because of the process, the color of black tea brewing water is typically yellow, deep yellow or reddish yellow, oolong and green tea brewing water is light green-yellow to greenish yellow. In the experiment, the color of brewing water from black and green tea was exactly as expected, but the color of oolong brewing water distributed in a wider range from greenish yellow to reddish yellow, because of partly oxidized catechin during processing.

3.2. Total polyphenols and total catechin contents



1- 4 : Oolong tea 5 – 8 : black tea 9 – 27 : green tea

Figure 3.1. Total polyphenols and total catechins contents in tea products

Table 3.2. Distributional range and mean value of total polyphenols and total catechins of tea products

Total polyphenols (ppm GE)

Tea types	Minimum	mean	Maximum
Oolong tea	12.37	13.12±0.53	14.02
Black tea	11.39	12.40±0.18	14.00
Green tea	5.56	15.06±2.12	18.69

Total catechins (ppm CE)

Tea types	Minimum	mean	Maximum
Oolong tea	4.94	5.29±0.55	6.39
Black tea	1.52	1.70±0.14	1.94
Green tea	1.45	7.33±1.71	9.86

Green tea had the highest TPC (15.06 ± 2.12 ppm GE), higher than oolong tea (13.12 ± 0.53 ppm GE) and black tea (12.40 ± 0.18 ppm GE). It should be noted that Folin agent in analysis procedure reacted with all phenolic compounds, it is impossible to distinguish between virgin catechins and oxidized products. Consequently, total polyphenols content in all three types of tea were not significantly different. However, the results varied in a wider range in case of total catechins contents. TCC of green tea (7.33±1.71 ppm CE) was higher than TCC of oolong tea (5.29±0.55 ppm TCC) and exceeded that of black tea (only 1.70±0.14 ppm CE) (Table 3.2).

Although the results showed convincingly the trend of TPC and TCC in three types of processed tea, it revealed a problem in quality of green tea. From the nature of green tea process, green tea was expected to have the TPC value not surpass TCC value, which means less catechins compounds was oxidized during processing [2, 15]. From the figures in Table 3.2, TCC of green tea was roughly half of TPC, yet still higher than oolong tea (40%) and black tea (14%). This results was appropriate for oolong and black tea, however indicated low quality of green tea products. In all 27 kinds of green tea in this studies, five (No. 10, 15, 22, 23, 26) had TPC and three (No. 22, 23, 26) had TCC lower than those of oolong tea, No. 26 even had TCC and TPC lower than those of black tea.

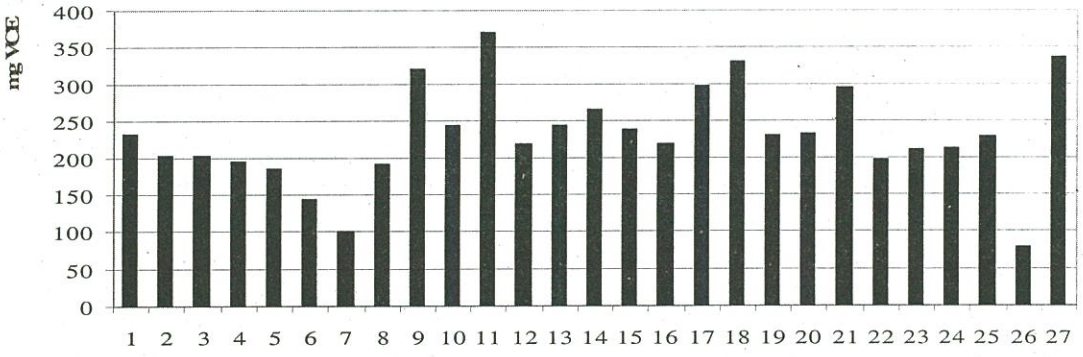
Table 3.3. Percentage of polyphenols and catechins in the first fusion versus total polyphenols and catechins content

Tea types	Polyphenols in first fusion	Total polyphenols	Percent	Catechin in first fusion	Total catechin	Percent
Oolong tea	5.51±0.49	13.12±0.53	42	2.49±0.28	5.29±0.55	47
Black tea	5.33±0.56	12.40±0.18	43	1.06±0.07	1.70±0.14	62
Green tea	6.80±1.29	15.06±2.12	45	3.30±0.69	7.33±171	45

Interestingly, it was observed that only around 45% of polyphenols and catechins content in tea product was extract in first brewing water (black tea had highest extractable catechin (62%) in the first brewing water). In experiments, it was necessary to repeat brewing procedure at least three times to obtain all polyphenols and catechins from tea products.

3.3. DPPH free radical scavenger activity

DPPH free radical scavenger activity of the first infusion brewing water were measured, compared with Vitamin C and shown below as Vitamin C equivalent.



1- 4 : Oolong tea 5 – 8 : black tea 9 – 27 : green tea

Tea types	Minimum	mean	maximum
Oolong tea	95	208±11	232
Black tea	99	155±33	191
Green tea	78	288±23	369

Figure 3.2. DPPH radical scavenger activity of teas fusion

Similarly to the results of TPC and TCC, green tea had the highest RSA (288±23 mg Vitamin C), higher than that of oolong tea (155±33 mg Vitamin C) and black tea (155±33 mg vitamin C). The DPPH RSA of tea was attributed to their phenolic constituents. Catechin monomers such as catechin, catechin gallates, epicatechin... which were abundant in green and oolong tea have stronger antioxidant activity than oxidized products [3, 15]. It was found that EC50 value have a good correlation with TPC (r^2 = 0.81) and TCC (r^2 = 0.77) (Figure 3.3), also indicated that others phenolic compound in tea may contribute to the total RSA of the first brewing water of tea products [11, 15].

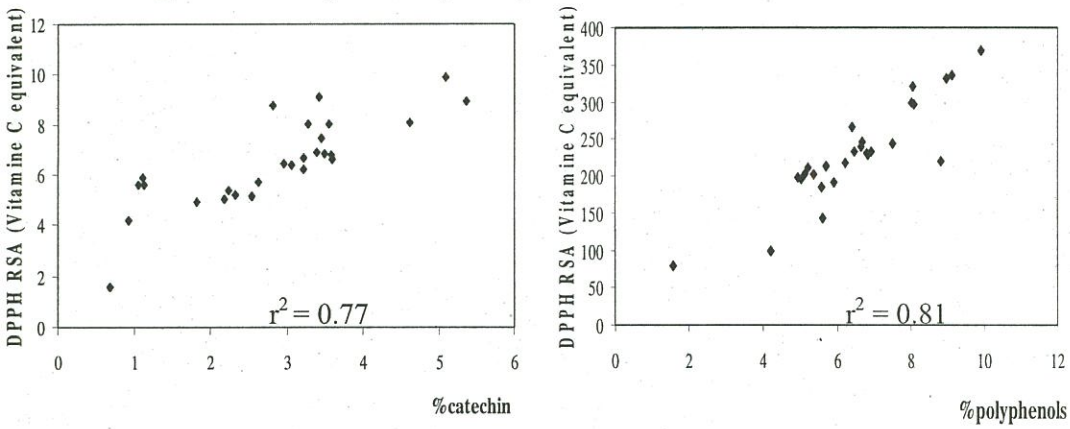


Figure 3.3. Relationship of catechins and polyphenols content to DPPH RSA

4. CONCLUSION

This article reports useful data about the total polyphenols, total catechins content and free radical scavenger activity of 30 types of green, oolong, and black tea in Vietnam market. Green tea exhibited the highest mean value of TPC, TCC and RSA, which make green tea as a good antioxidant source for consumer. However, the results showed the instability and unevenness of quality among 27 type of investigated green tea. Because of green tea production have several steps and affect by many factors (the process itself, tea breed, soil or even the way famers cultivate tea shoots...), it needs further research to investigate and confirm this observation, as well as locate the reason, and find the solution for production of high quality green tea.

HÀM LƯỢNG POLYPHENOLS TỔNG, CATECHIN TỔNG VÀ HOẠT TÍNH QUÉT GỐC TỰ DO DPPH CỦA MỘT SỐ LOẠI TRÀ XANH TRÊN THỊ TRƯỜNG VIỆT NAM

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TÓM TẮT: *Ba mươi loại trà nhãn hiệu Việt Nam khác nhau (21 loại trà xanh, 5 loại trà đen và 4 loại trà oolong) trên thị trường Thành phố Hồ Chí Minh được khảo sát về hàm lượng polyphenol tổng (TPC – tính theo acid gallic), catechin tổng (TCC – tính theo catechin) và hoạt tính quét gốc tự do DPPH (RSA – tính theo vitamine C). Trong nhóm khảo sát, trà xanh có hàm lượng polyphenol, catechin tổng cao nhất (TPC 15.06 ± 0.53 ppm, TCC 7.33 ± 1.71 ppm) và hoạt tính quét gốc tự do lớn nhất (RSA 288 ± 23 mg) trong 3 gam trà khô thành phẩm, các giá trị thu được cao hơn trà oolong, trong khi trà đen ở vị trí thấp nhất. Tuy nhiên, một số loại trà xanh cho các kết quả thấp hơn mong đợt, thậm chí thấp hơn kết quả của trà đen. Kết quả này cho thấy sự không ổn định và đồng đều của chất lượng trà xanh trên thị trường Việt Nam.*

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