MICROWAVE-ASSISTED EXTRACTION OF POLYPHENOLS FROM FRESH TEA SHOOT

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ABSTRACT: *Fresh tea shoots (one shoot and two or three young leaves) were extracted using microwave-assisted extraction method (MAE). Several factors such as ethanol concentrations (0-99.5° (volume percentage)), material: solvent ratio (1:4 to 1:12), extraction time, oven power were studied to optimise conditions at laboratory scale. MAE archived good yield (82.46 %) after 6 minutes microwave radiation, that was higher than that of extraction at room temperature in 24 hours, conventional heating extraction at reflux temperature in 60 minutes or ultrasound-assisted extraction in 60 minutes. Afterward, MAE method was employed to investigate five types (LD97, LDP1, PH1, TB14, HAT) of tea (Camellia sinensis (L.) O. Kuntze) from Bao Loc, Lam Dong. HAT (20.49 % dried weight of total catechins) was found as suitable material for polyphenols extraction.*

Keywords: *Polyphenols, catechins, tea extract, extraction, microwave-assisted extraction.*

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. Recently tea has attracted scientific attention for its anticancer and antioxidant activities [10]. Polyphenols in green tea are believed as excellent free radical scavengers. Several clinical studies have proved polyphenols to be active in cancer prevention in several ways. Polyphenols have also been recently recognized as functionally active molecules, possessing antioxidant, anticancer, anti-mutagenic properties, as well as exerting protective effects against cardiovascular and other diseases [13,15].

This is primary because of the affect of polyphenols, tea main components, which may be as high as 30 – 35 % in dried weight of fresh tea shoots [2, 18, 20]. 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....[3, 8, 20]. 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) [16].

Conventional methods for extraction of tea polyphenols have been reported [12, 17, 19]. Microwave-assisted extraction, which has been recently successfully used for extract biologically active compounds [11, 14], has been studied for extraction polyphenols from tea. There is already a report done on the use of MAE for extraction of tea polyphenols [8]. Commercial dried green tea was used as material for extraction, and higher yield was achieved in shorter time in MAE compare to those of conventional methods. However, dried green tea, which require long process and is an expensive material, may not suitable for polyphenols extraction at large scale.

Nowadays, tea extract and polyphenols from tea have become common products in several countries such as China, Japan, Indian...However, tea extract is still new in Vietnam market and also new in research and application. The purpose of this work is to evaluate MAE method

for extracting polyphenols from tea and apply this method to investigate several tea resources in Bao Loc, Lam Dong.

2. MATERIALS AND METHODS

2.1. Materials

Fresh tea (picked as shoots with two or three leaves) from Bao Loc, Lam Dong, was kindly provided by Minh Rong Tea Company. Tea was inactivated enzyme by steaming for 90 seconds before using.

Folin - Ciocalteu agent (Merck), (+)-catechin, (-)- epicatechine, (-)-epicatechin gallate, (-)-epigallocatechin gallate, caffeine (98% HPLC, Aldrich – Sigma), ethanol and other chemical were analytical purity grade.

2.2. Microwave assisted extraction (MAE)

Experiments were carried out in a domestic microwave oven (Whirpool Oven, power 450, 600, 800 W), which was modified for using in laboratory [1]. 100 g of tea (which had been inactivated enzyme before) were cut and grinded to small size, then mixed with solvents in suitable ratio. The suspension was radiated in microwave oven at regular intervals (one minute radiation and two minutes off) to keep temperature not rise above 80°C. The infusions were let to cool down to room temperature, filtered and stored in refrigerator at 4°C for determine total polyphenols later.

Other extraction method in comparison included conventional heating reflux extraction method, ultrasound-assisted extraction and extraction at room temperature.

2.3. Total polyphenols determination

Folin-ciocalteu method, is one of several methods which are used to determine total polyphenols [6, 20]. Polyphenols reduce Folin agent (yellow solution of polyphosphatetungstenate and molydate) in mild base medium to form deep blue color. The procedure was modified from [6], 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 725 nm 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)

2.4. Catechins determination

Catechins were measured using HPLC method described in previous literature with modified [3, 5, 7, 17]. Analyses were performed on an HPLC 1100 Agilant, with C18 column (Synergi Hydro-RP 80R, Phenomenex, US) and UV detector (230 nm). Eluent A was acetonitril (ACN) and eluent B was 0.1 % aqueous phosphoric acid, the flow rate was kept constant throughout the analysis at 1 ml. min^{-1}. The elution programme was used as following:

Time (minute)	:	0	10	10.1
ACN	:	5	40	5
H_3PO_4 (0.1 %)	:	95	60	95

Chromatograms were monitored at 230 nm and identification was based on retention times in comparison with authentic standards. Quantification was performed by establishing calibration curves for each compound determined, using standards.

3. RESULTS AND DISCUSSION

3.1. Microwave-assisted extraction

3.1.1. Effect of ethanol concentration on the extraction of polyphenols

Ethanol was employed in experiment because it is non-toxic and inexpensive solvent. Results on figure 1 showed that the extraction of polyphenols from tea was influenced by ethanol concentration in water. When ethanol concentration increased from 0 to 60°, polyphenols content in the extract increased significantly. Further increase of ethanol concentration was only affected in slightly increase of polyphenols content. This result contradicted with that of [8], the extraction was decreased with the increase of ethanol concentration.

Besides polyphenols content, the color of extracts changed from greenish yellow to deep green color when the volume of ethanol percentage was higher than 60°, which indicated that more undesirable compounds, primary chlorophyll, were also extracted. Therefore, it was decided to use ethanol 60° as extraction sovent in the following experiments.

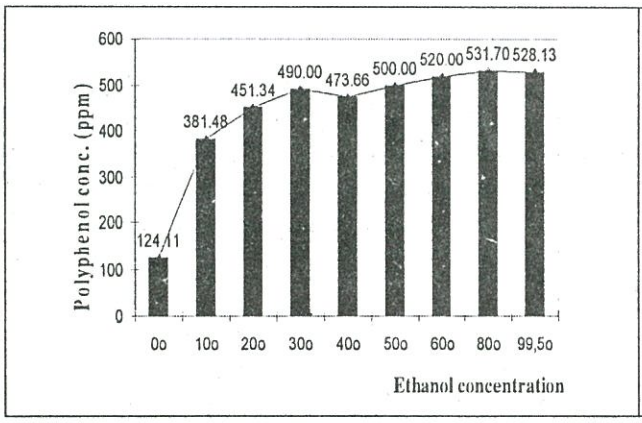

Figure 1. Effect of ethanol concentration on the extraction of polyphenols. Material: solvent ratio = 1:6 (g/ml), solvent volume 150 ml, radiation time: 6 minutes, 800 W power

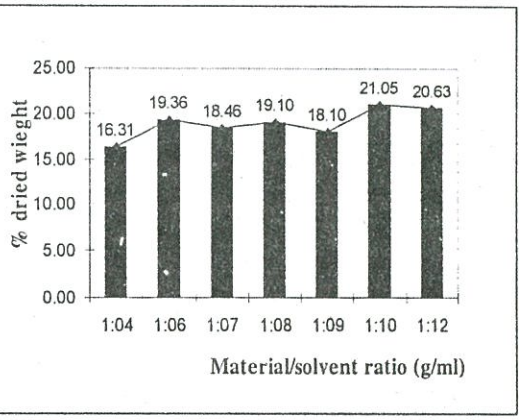

Figure 2. Effect of material/solvents ratio on the extraction of polyphenols. Solvent ethanol 60°, volume 150 ml, radiation time: 6 minutes, 800 W power.

3.1.2. Effect of material/solvents ratio on the extraction of polyphenols

It was found that material/solvent ratio did not have much effect to yield of the extraction. Figure 2. showed that the extraction was increased with the increase of material/ solvent ratio from 1:4 to 1:6, afterward, yield of extraction was slightly decreased, from 19.36 to 18.10 with ratio increased from 1:6 to 1:8, then increased, from 18.10 to 21.06 with ratio 1:10. Using high ratio of material/solvent could help achieve high yield of extraction (i.e. 1:10), however large amount of solvent resulted polyphenols content in the extract was unnecessary low. Therefore, material/solvent ratio of 1:6 was sufficient for the experiment. This ratio was much lower than suitable ratio in [8] (1:20), it could be explained that material used in this experiment was fresh tea shoot with 75 – 80 % humility, while as material used in [8] was dried green tea (humility usually from 5 to 7 %).

3.1.3.Effect of radiation time on the extraction of polyphenols

Polyphenols concentration in the extracts was rapidly increased in the first 6 minutes of extraction (Figure 3). Further radiation was only slightly increased polyphenols concentration. Hence it was unnecessary to carry experiments with more than 6 minutes radiation (In [8], it took only 4 minutes to obtained highest level of polyphenols concentration in extract). It was also found that using higher oven power could achieved better yield with shorter times (4

minutes at 800 W gave similar yield as 6 minutes at 450 W) (In [8], microwave oven with full power 700 W was employed in experiments).

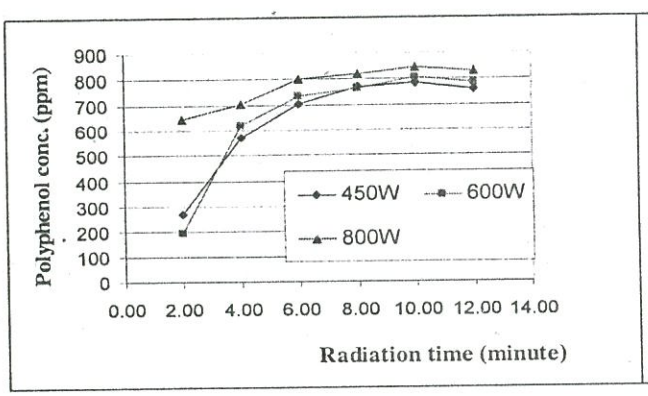

Figure 3. Effect of radiation time on the extraction of polyphenols. Solvent: ethanol 60° volume 150 ml, material: solvent ratio 1:6 (g/ml)

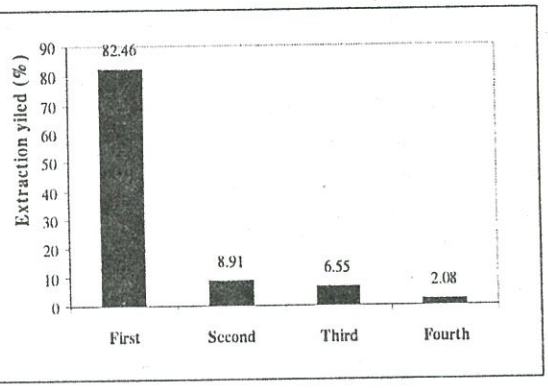

Figure 4. Yields of extraction in consecutive times. Solvent: ethanol 60°, volume 150 ml, material: solvent ratio 1:6 (g/ml), radiation time: 6 minutes, 800 W power.

3.1.4. Yields of extraction in consecutive times

This procedure was repeated several times until water did not change color when adding drops of indicator solution $FeCl_3$ 3 % / HCl 0.1 N (dark blue color appears if there are polyphenols in water) to extract all polyphenols in tea material. Figure 4 shows that yield in the first extraction (82.40 %) is much higher than that of following extraction (8.91 %, 6.55 %, 2.06 % in the second, third and fourth time, respectively). This result was demonstrated high yield of MAE method.

3.1.5. Comparison of the results of extraction with MAE and other methods

Other extraction procedures (conventional heating reflux extraction method, ultrasound-assisted extraction and extraction at room temperature) were carried out for comparison to MAE. Results in table 1 showed that yield (82.46 %) of MAE for 6 minutes radiation (18 total time of extraction) higher than that of ultrasound-assisted extraction in 60 minutes, conventional heating reflex extraction in 60 minutes and extraction at room temperature in 24 hours (63.3 %, 65.64 % and 49.39 %, respectively).

Table 1. Comparison of the results of extraction with MAE and other methods

Method	Time	Catechins	Yields(%)
MAE	6 minutes	16.90 (% dried weight)	82.46 %
Conventional heating reflux	60 minutes	13.45	65.64
Ultrasound-assisted	60 minutes	12.97	63.30
Extraction at room temperature	24 hours	10.12	49.39

Solvent : ethanol 60°, volume 150 ml; material : solvent ratio 1:6 (g/ml); MAE : radiation time 6 minutes, 800 W power; conventional heating reflux extraction at boiling point, using magnetic stirrer; room temperature : 30° C; ultrasound-assisted at 30° C.

3.2. Catechins content in tea

3.2.1. HPLC determination of catechins in tea extract

To investigate tea extract, caffeine and four catechins were chosen, their content were determinated by reversed-phase HPLC. In experiment, the peaks correspondent to catechin (C), epicatechin (EC), epicatechin gallate (ECG), epicagallocatechin gallate (EGCG) and caffeine

could be separated, identified and quantified. In HPLC chromatograph, besides five peaks already identified, several minor peaks were also detected, which indicated other catechin compounds in extract. It was found that the similarity of HPLC chromatographs indicated the similarity in catechin components in all five tea breeds in Bao Loc, Lam Dong.

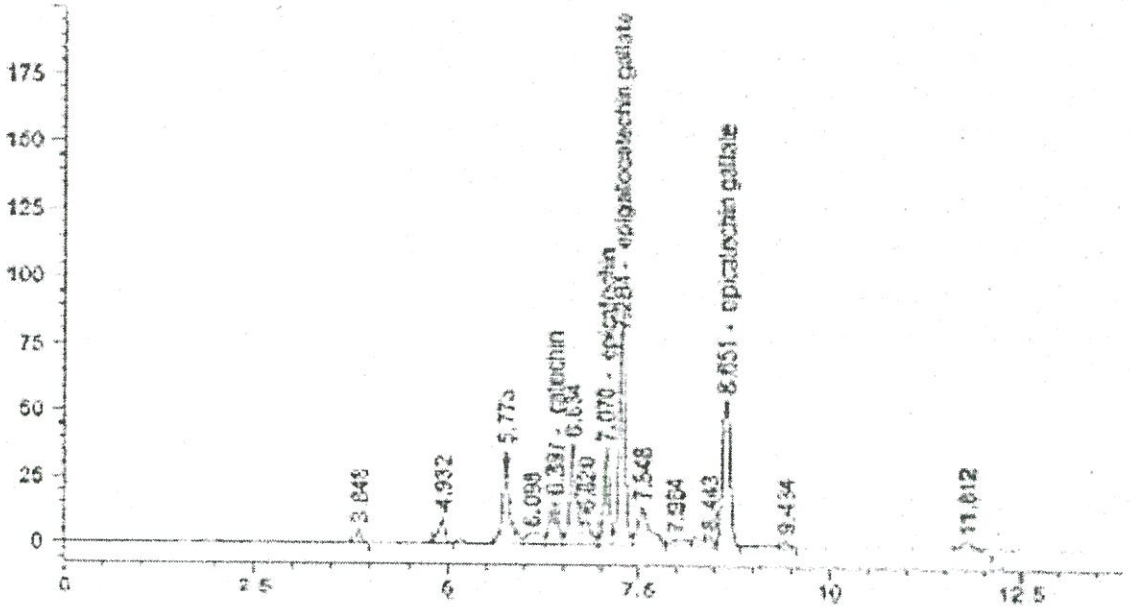

Figure 5. Typical HPLC trace of tea extract, recorded at 230 nm. Peak assignment: 6.331, catechin; 6.634, caffeine; 7.070, epicatechin; 7.264, epigallocatechin gallate; 8.651, epicatechin gallate.

3.2.2. Comparison of tea resources

The results showed that HAT tea had the highest amount of catechin compounds (C + ECG + EC + EGCG = 20.49 %) higher than that of LD97, PH1, LDP1, TB14 (14.32 %, 16.09 %, 16.32 % and 15.26 % respectively). Therefore, HAT tea should be used as material for extraction.

Among four catechin compounds identified, the most abundant catechin compound was epigallocatechin gallate (which accounting for 80 % of total catechins in HAT, PH1 and LDP1, 65 % in LD97 and TB14, followed by epicatechin gallate (around 12 – 18 % of total catechins) and epicatechin (7 -12 %). By constract, catechin was only minor constituent (6 % in LD97, 2.4 % in PH1, 2.6 % in TB14, 0.9 % in HAT, 0.5 % in LDP1).

It was also observed that caffeine distributed evenly among five tea origins (from 2.5% to 3.3%). In constract with catechins, caffeine amounts may not depend on tea origins or conditions of the experiments.

Table 2. Analytical catechin compounds and caffeine of tea extracts

Tea breeds	Catechin (% dried weight)					Caffeine (%dried weight)
	C	EC	EGCG	ECG	Total	
LD97	0.87	1.78	9.11	2.56	14.32	2.8
PH1	0.39	0.93	12.85	1.92	16.09	3.3
HAT	0.19	1.52	16.30	2.48	20.49	3.2
LDP1	0.08	1.03	13.57	1.63	16.32	2.5
TB14	0.41	1.94	10.09	2.82	15.26	3.1

Solvent : ethanol 60°, volume 150 ml; material : solvent ratio : 1:6 (g/ml); MAE : radiation time : 6 minutes, 800 W power.

4. CONCLUSION

Conditions of MAE method were studied and established. MAE method was proven as more effective than other conventional methods for extracting polyphenols from tea. MAE method provided higher yield in shorter times (82.4 % after 6 minutes radiation).

MAE method was employed to extract five type of tea from Bao Loc, Lam Dong. HPLC analysis of the extract showed the similarity of catechin compounds in all five tea origins, in which EGCG and ECG were the main components. HAT tea, with the highest amount of EGCG and total catechins, could be used as suitable material for extraction

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TRÍCH LY CÓ HỖ TRỢ VI SÓNG CÁC POLYPHENOL TỪ BÚP TRÀ TƯƠI

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TÓM TẮT: *Phương pháp trích ly có hỗ trợ vi sóng được sử dụng để trích ly polyphenol từ búp trà tươi (búp và 2, 3 lá non). Các thông số liên quan như nồng độ ethanol (0-99.5° (% theo thể tích)), tỉ lệ nguyên liệu : dung môi (g/ml), thời gian trích ly, công suất lò vi sóng được khảo sát để xác định điều kiện thích hợp cho quá trình trích ly polyphenol từ trà. Phương pháp trích ly có hỗ trợ vi sóng thu được hiệu quả trích ly tốt (82.46 % sau 6 phút chiếu vi sóng), cao hơn phương pháp trích ly bằng gia nhiệt thông thường trong 60 phút , phương pháp trích ly có hỗ trợ siêu âm sau 60 phút hay phương pháp ngâm trích sau 24 giờ. Sau đó, phương pháp trích ly có hỗ trợ của vi sóng được sử dụng để khảo sát 5 loại nguyên liệu LD97, LDP1, PH1, TB14, HAT trà (Camellia sinensis (L.) O. Kuntze) từ vùng trà Bảo Lộc, Lâm Đồng. Giống trà HAT (có hàm lượng catechin cao nhất 20.49 % khối lượng khô) thích hợp làm nguyên liệu trích ly.*

REFERENCES

[1]. Andre Loupy, Tran Kim Quy, Le Ngoc Thach, *Phương phap hoc moi ve tong hop huu co*, HCM City University of Natural Science, Viet Nam, 1995.

[2]. Tong Van Hang, *Co so sinh hoa va ki thuat che bien tra*, HCM City, Viet Nam, 1985.

[3]. Lawrance Peter Wright, *Biochemical analysis for identification of quality in black tea (Camellia sinensis)*, Doctoral Thesis, University of Pretoria, South Africa, 2005.

[4]. Zohar Kerem, Hilla German-Shashoua, Oded Yarden, *Microwave-assisted extraction of bioactive saponins from chickpea (Cicer arietnum* L.*)*, J. Sci. Food Agric. 85, 406 – 412, 2005.

[5]. Joung Ho Ko, Gyoung Won Kang, You Jin Seo, Won Jo Cheong, *C18 attached silica monolith microcolumns made in stainless steel tubing and their application in analysis of flavonoids in green tea extracts*, Bull. Korean Chem. Soc. 25 (10), 2004.

[6]. Jan Wollgast, *The contents and effects of polyphenols in chocolate, qualitative and quantitative analys of polyphenols in chocolate and chocolate raw products as well as evaluation of potential implications of chocolate consumption in human health*, Doctoral thesis, Jutus Liebig University of Giessen, Germany, 2004.

[7]. Kai On Chu, Chi Chiu Wang, Micheal Scott Rogers, Kwong Wai Choy, Chi Pui Pang, *Determination of catechins and catechin gallates in biological fluids by HPLC with colormetric array detection and solid phase extraction*, Analytica Chimica Acta 510, 69 – 76, 2004.

[8]. Xuejun Pan, Guoguang Niu, Huizhou Liu, *Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves*, Chemical engineering and processing 42, 129 – 133, 2003.

[9]. Carmen W. Huie, *A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants*, Anal. Bioanal. Chem. 373, 23 – 30, 2002.

[10]. Hirota Fujikia, Masami Suganumab, Kazue Imaic, Kei Nakachic, *Green tea: cancer preventive beverage and/or drug,* Cancer Letters 188, 9 –13, 2002.

[11]. E.Surducan, Camellia Neamtu, V. Surducan, Gabiela Nagy, S.Filip, *Microwave power assisted sample preparation, extraction studies*, Studia Universitatis Babes-Bolyai, Physica, Special Issue, 2001.

[12]. David T. Bailey, Parh L. Yuhasz, Bo Lin Zheng, *Method for isolation caffeine –free catechins from green tea*, US patent 6.210.679, 2001.

[13]. John H. Weisburger, *Tea and health: a historical perspective,* Cancer Letters 114, 315 - 317, 2000.

[14]. M.Letellier, H. Budzinski, *Microwave-assisted extraction of organic compounds*, Analysis (Wiley – VCH) 27, 258 - 271, 1999.

[15]. Michael D. Brown, Green *Tea (Camellia Sinensis) Extract and Its Possible Role in the Prevention of Cancer*, ND Alternative Medicine Review, Vol. 4 (5), 1999.

[16]. Qiong Guo, Baolu Zhao, Shengrong Shen, Jingwu, Jungai Hu, Wenjuan Xin, *ERS study on the structure – antioxidant activity relationship of tea catechins and their epimers*, Biochimia et Biophysica Acta 1427, 13 - 23, 1999.

[17]. Yuko Yoshida, Masaaki Kiso, Tetsuhisa Goto, *Efficiency of the extraction of catechins from green tea,* Food Chemistry 67, 429 - 433, 1999.

[18]. Edwin Haslam, *Plant polyphenols,* Cambridge University Press, 1992.

[19]. Yukihiko Hara Shizuoka, *Process for the production of tea catechins*, US patent 4.613.672, 1986.

[20]. T.A. Geissman, *The Chemistry of Flavonoid compounds*, New York, USA, 1962.