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Recycling of Tea Waste: Simple and Effective Separation of Caffeine and Catechins by Microwave Assisted Extraction (MAE)

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Abstract: This study was designed to develop a cost effective green extraction method to separate valuable chemicals from black tea waste (BTW) and tea dust called "caffeine dust" (CD). These materials are totally unwanted residue and treated as rubbish. Two extraction methods were used for extraction of waste materials obtained in three collection periods. In conventional extraction method, material was subjected to hot water extraction at 80 ºC for 40 minutes. In microwave assisted extraction (MAE) method, different eco-friendly aqueous solvents (water, citric acid-water or ethanol-water) were used under a controlled 600 W microwave power at 80 ºC for only 4 minutes treatment in a close microwave system. MAE and conventional method gave similar extract yields for both waste materials but MAE was more successful and economical completing the extraction in shorter time. Using citric acid-water solvent in MAE significantly increased the extraction of individual catechins such as epicatechin (EC) and epigallocatechin gallate (EGCG). This study showed that tea waste could be utilized for production of valuable chemicals using nontoxic solvents at extremely short extraction periods.

Keywords: Tea waste, caffeine, catechins, microwave assisted extraction,

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1. Introduction

Black tea is one of most favored drink in all societies. Different methods are used for black tea production to obtain its red bright infusion. Basic steps for production line are withering, rolling, fermentation and drying. Among them, fermentation step is the most significant process of making black tea since it defines the quality of the tea.

In Turkey, tea is planted in Eastern Black Sea region and harvested in three collection periods; first collection is in May, second is in June, and the third is in August-September. Almost half of black tea is processed by the government based tea processing plants in seven grades [1]. All tea factories use there main techniques namely orthodox, rotorvan and CTC (curling, tearing, crushing) or combination of these techniques. Çaykur is a government based company and use either orthodox (nonpressing orthodox+sieving+pressing orthodox) or modified Çaykur method (nonpressing orthodox+rotorvan+sieving+conical orthodox). During these processes tea leaves are broken down to small particles and this is not desired by tea producers. Small particles are separated by sieving or air flow from black tea that has a market value. This waste called "caffeine dust" and produced at large quantities. It is totally unwanted

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residue and treated as rubbish or burned for energy production. Another waste material is also produced during production of black tea. This material is different than caffeine dust and mainly contains cellulosic residue of tea stalks. Çaykur sells 200.000 tones waste material in 2014 and guaranties that they will supply 20.000 tons/year for next 10 years. According to new legislation in Turkey damping these waste materials are not allowed and it must be consumed in an appropriate way. Burning is one of the possible option but created massive air pollution.

On the other hand both black tea waste (BTW) and caffeine dust (CD) contain the same compounds as black tea has: tea polyphenol, caffeine, amino acids, saponins, tannins, etc., [2]. Tea polyphenols are catechins, flavanols, phenolic acids, flavanonens, glycosides and plant pigments [3] . The catechins can be categorized into two groups based on their structure: epistructured catechins and non-epistructured catechins. The epistructured catechins are epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG), of which EGCG is the major constituent and the most powerful one. Non-epistructured catechins are gallocatechin (GC), catechin (C), gallocatechin gallate (GCG), and catechin gallate (CG) [4]. This group of compounds is known responsible from anticarcinogenic and antimutagenic properties of tea, as well as protective action against cardiovascular diseases. They are good antioxidant agents and have been used to enhance the shelf-life of food products [5]. Therefore, these waste materials can be utilized for production of valuable chemicals such as caffeine and catechins. But an economical and eco-friendly extraction method should be used for separation of these chemicals from tea waste. Extraction time and consumed energy is an important issue to process the waste materials at large quantities.

Conventional solid-liquid extraction is one of the methods that are used for effective extraction of caffeine and catechins from tea samples. Many different treatment steps were used for extraction of caffeine and catechins that mainly consists hot water as extracting solvent [6-12].

Other methods have been proposed for effective extraction of chemicals from tea. A review published by Vuong et al. (2011) contains all important techniques for the extraction of tea samples. Although hot water extraction methods are quite simple and easy they require long extraction periods and energy that are not feasible for large scale production. Separation of valuable chemicals from tea waste at large quantities need sophisticated techniques that: *i*) has relatively lower and economical extraction periods *ii)* provide high extract yields and *iii*) selective to target compounds.

Microwave assisted extraction (MAE) is a new techniques that requires lower extraction period and less energy consumption. Microwave power produces high temperature regions that ease the disruption of the cell wall and facilitates the extraction. As a result of these effects caffeine and catechins are readily extracted into the extracting solvent [2,13]. Recently Nkhili et al. (2009) has reported a study for extraction tea using a 600 W microwave oven. The temperature was controlled between 80 and 100 ^{o}C for 30 min and the solvent to tea ratio was 20:1 (ml/g). Under these conditions the extraction efficiency for the tea catechins was reported to be higher than for the conventional hot water methods. Therefore, MAE of tea waste might be a good approach to meet all requirements.

The objective of this study is to establish MAE methods for cost effective extraction of caffeine and catechins from black tea waste (BTW) and caffeine dust (CD). Two extraction methods, conventional hot water and microwave assisted extraction (MAE), were employed and different extracting solvents were tested for MAE. Waste material was collected from the same tea factory in different collection periods and subjected to extraction. After successive separation of caffeine and catechins from extraction medium these constituents were quantified by chromatographic analyses to determine the amount of individual catechin. The results of this study can be used as reference for recycling of these waste materials for cost effective production of valuable chemicals. This is the first report employing MAE for extraction, isolation and quantification of industrial important chemicals from waste materials such as caffeine dust.

2. Material and Methods

2.1. Standards and chemicals

Methanol, ethanol, ethyl acetate and chloroform were analytical grade from Merck. The standard chemicals of (−)epigallocatechin (EGC), (−)epicatechin (EC), (−)epigallocatechin gallate (EGCG), (−)epicatechin gallate (ECG) and caffeine were purchased from Sigma (St Louis, MO, USA). Black tea waste and caffeine dust used in the experiments were collected from Sürçay San. Co. Ltd. (Sürmene, Trabzon, Turkey). All experiments works were repeated three times.

2.1.2. Conventional hot water extraction

Briefly, 10 g of waste material was extracted with 200 ml of pure water at 80 °C for 40 min. Residue was removed by filtration using a filter paper (pore size: 5 µm) and filtrates were initially partitioned with chloroform to remove caffeine. Then aqueous phase was extracted with ethyl acetate three times using 150 ml ethyl acetate in each extraction to separate catechins. The chloroform and ethyl acetate phases were filtered and the filtrate was concentrated by a rotary evaporator under reduced pressure at 55 °C.

2.1.3. Microwave assisted extraction (MAE)

Tea catechins and other components were extracted using a close microwave assisted extraction system (MILESTONE, START S Microwave, USA). MAE parameters such as microwave power, extraction time and extracting solvent can affect the efficiency [14]. 10 g of sample and 200 ml extracting solvent were placed in vessel and soaked for 90 minutes at room temperature. Presence of citric acid or ethanol facilitates the selective extraction of catechins [4]. Therefore, three extracting solvents (water, citric acid-water or ethanol-water) were used. Citric acid-water solvent was prepared using 0.1 M citric acid and water (1:1). Ethanol-water was prepared diluting technical ethanol (96%) with equal volume of water. Then sample was transferred into microwave extraction apparatus [3]. Extraction was carried out under a controlled 600 W microwave power at 80 ^{o}C temperature for 4 min irradiation time [15]. After extraction, the flask was allowed to cool down room temperature before opening the cap. Aqueous infusion was extracted with first chloroform then ethyl acetate as described above.

2.1.4. Quantification of caffeine and catechins

In conventional and MAE quantification of caffeine and catechins were carried out by weighting the chloroform and ethyl acetate fractions after evaporation the solvent and drying the extract. Extract yields were calculated from the mass of extracts. HPLC analyses were carried out to determine the amount of each catechins present in the mixture [16]. The instruments used in this study was a HPLC system (Hitachi Elite Lachrom) equipped with a Shim-pack VP-ODS C18 column (5 mm, 4.6 x 250 mm, 35 °C) at 278 nm. Solvents A (water) and B (DMF-methanol-acetic acid mixture, 20:1:0.5) were run with 86% A for 13 minutes then its volume was decreased to 64% within next 15 minutes and finally back to initial concentration for another 6 min. Concentrations of catechins were quantified by their peak areas against those of standards prepared from original compounds.

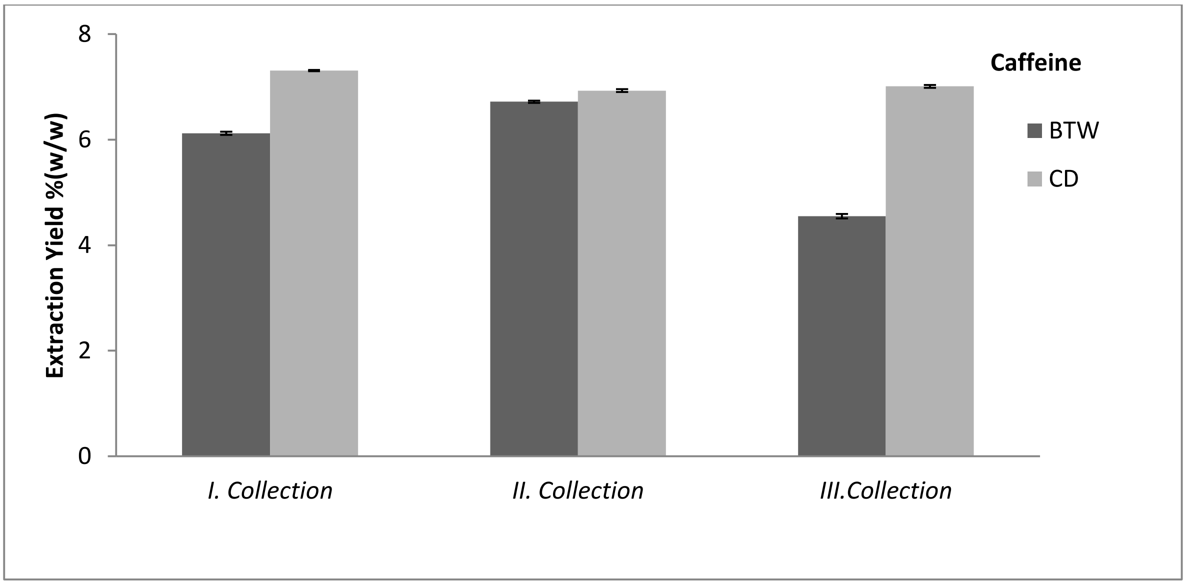
3. Results and Discussion

3.1. Extraction yields

3.1.1.Conventional Extraction

Waste materials were collected at three collection and processing times in 2013. The average caffeine and catechin yields from the first (in May, *I. Collection*), second (in June, *II. Collection*) and third collection period (in August-September, *III. Collection*) using conventional extraction method is given in Figure 1.

a)



b)

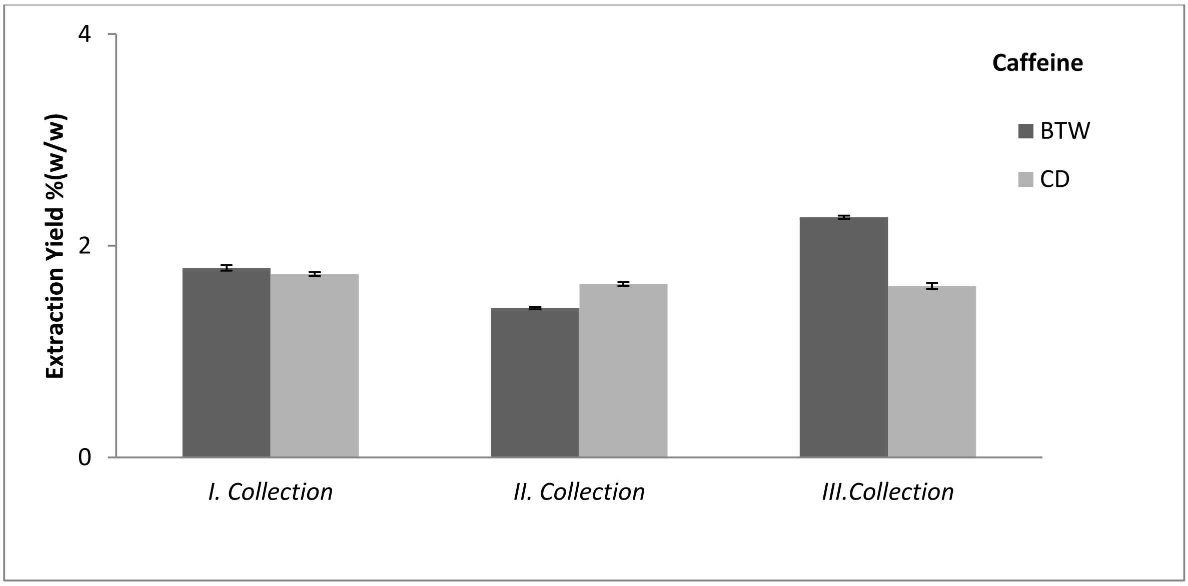


Figure 1. Caffeine and catechin yields of black tea waste (BTW) and caffeine dust (CD) in three collection periods. Data are expressed as the mean of three replicate.

Figure 1 shows that caffeine yields of both waste materials are quite high. Processed black tea usually contains 2-4% caffeine and Serpen et al. (2012) reported the caffeine content of black tea produced in the same region was between 1.5-1.8%. Their results were solely obtained from HPLC analysis and separation of caffeine from tea extract had not been carried out. In

BTW caffeine yields were similar at first two collection periods (6.12 and 6.72% respectively). BTW collected in the third collection period gave lower caffeine extract (4.25%). CD gave higher caffeine yields than BTW at all collection periods (ranging between 6.72-7.31%).

Although catechin content of BTW was slightly higher than CD both waste materials have lower catechin yields. However, catechin yield was slightly higher in BTW collected in the third collection period (2.27%).

3.1.2 Microwave Extraction of BTW and CD

Waste material was subjected to MAE in different solvents for a 4 minutes exposure period. Extract yields (%, w/w) obtained from black tea waste and caffeine dust is given in Figure 2 and 3.

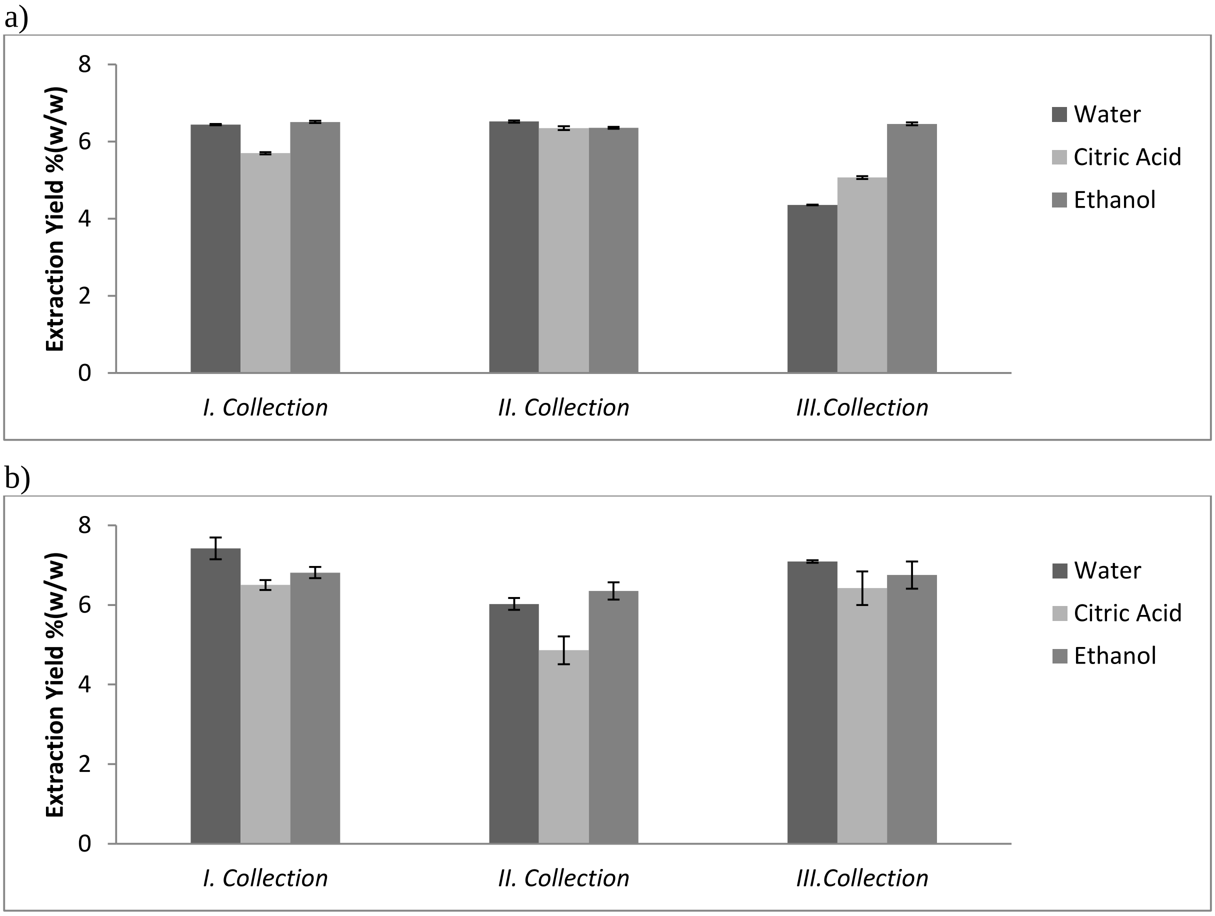


Figure 2. Caffeine yield of **a)** black tea waste (BTW) and **b)** caffeine dust (CD) in three collection periods. Data are expressed as the mean of three replicate.

First of all, caffeine yields in both waste materials were high as much as conventional hot water extraction. Extract yields varied between 4.36-7.42% in all collection periods and solvent systems. The mass of caffeine extracts was highest in CD reaching 7.42% (w/w, 74.2 mg/g tea waste). Caffeine content is usually between 2-4% in black or green tea that means 20-40 mg caffeine can be obttained from 1 gram of tea material [1]. In current literature there are published studies related to extraction of caffeine from green tea and tea waste. Water and supercritical carbon dioxide extraction (SFE) of caffeine from tea plant waste (mainly stalks) was reported by İçen and Gürü [12, 17, 18]. They reported that the maximum yield of caffeine from tea stalk wastes and fiber wastes were 14.95 mg/g and 18.92 mg/g, respectively. According to another study carried out by Shalmashi et al., (2010) supercritical water extraction of Iranian tea waste produced 0.77% (w/w) of caffeine while conventional hot water extraction yielded only 0.46% (w/w). Our results show that caffeine content of tea waste used in this study is extremely high and should be considered as a good source for caffeine extraction. Using water as extraction solvent seems to be more appropriate providing the same amount of caffeine in a 4 minutes extraction period for all collection periods. Citric acid-water or ethanol-water mixture does not alter the extraction yields except for third collection period of BTW. Using ethanol as co solvent in MAE increased the caffeine yield to 6.46%.

BTW and CD yielded similar amount of catechin extracts (Figure 3). The yields of BTW were between 1.41-2.27% for conventional extraction and 1.82-2.45% for MAE in water. Using citric acid-water or ethanol-water solvents slightly increase the catechin yields. It was the highest in the third collection period.

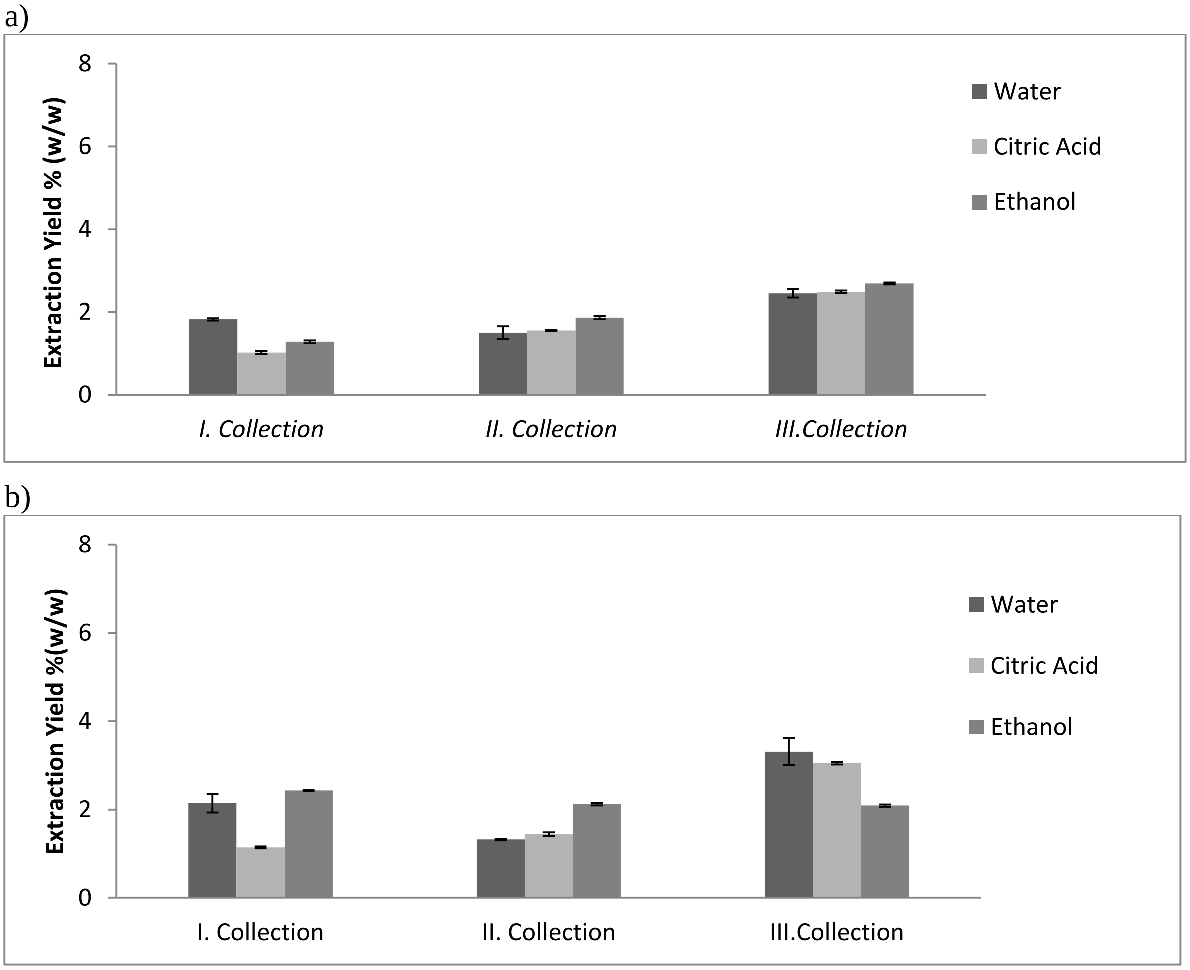


Figure 3. Catechin yield of **a)** black tea waste (BTW) and **b)** caffeine dust (CD) in three collection periods. Data are expressed as the mean of three replicate.

MAE of caffeine dust gave higher yields than conventional hot water extraction especially in third collection period. Ethanol-water solvent increased catechin yields in some collection periods but there was no constant improvement. But it is clear that extraction of catechins employing MAE is more promising since the same amount of extract was obtained after just 4 minutes treatment. Microwave seems to be facilitating the extraction of catechins into extracting solvent via heat zones formed in waste material (Oliveira and Franca, 2002). The temperature is localized in zones that ease the selective migration of target compounds from the material in shorter time.

Extraction yield calculated from extract mass can give a rough idea about how much caffeine and catechin can be isolated from extracting medium. Caffeine extraction with chloroform provides pure caffeine but separation of catechins by liquid-solid extraction is not purely selective and ethyl acetate can remove some other principles as well as catechins. Quantification of individual catechins present in the extract should be done for proper evaluation. Therefore, HPLC analyses were carried out to determine the real amount of each catechin present in the extracts.

3.1.3. HPLC analysis

Caffeine and catechin constituents of the extracts were determined by reversed-phase HPLC. A standard mixture is used for identification and quantification of individual catechin. HPLC chromatogram of standard mixture containing catechins, gallic acid and caffeine is given in Figure 4.

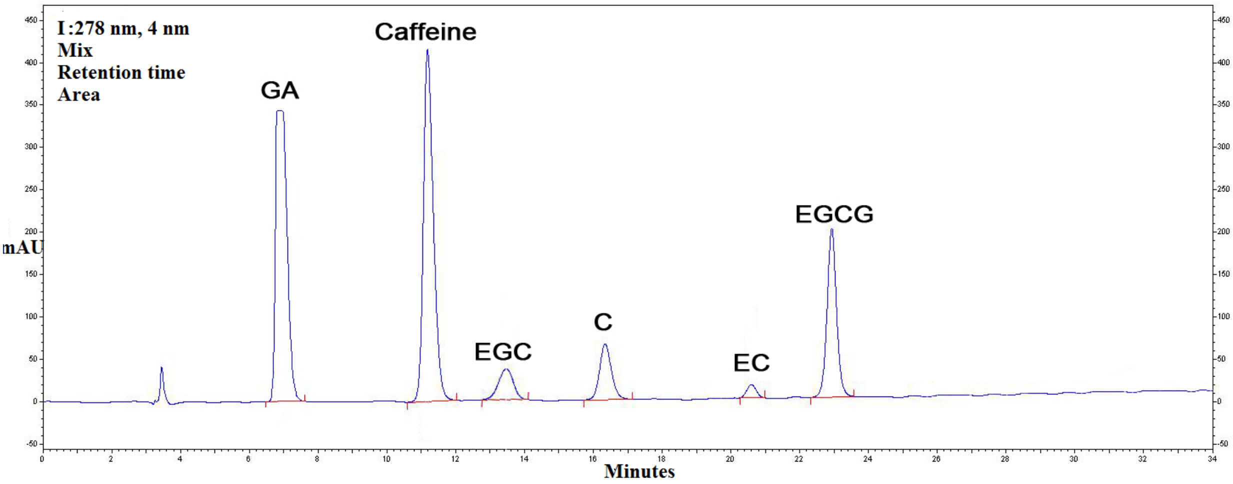
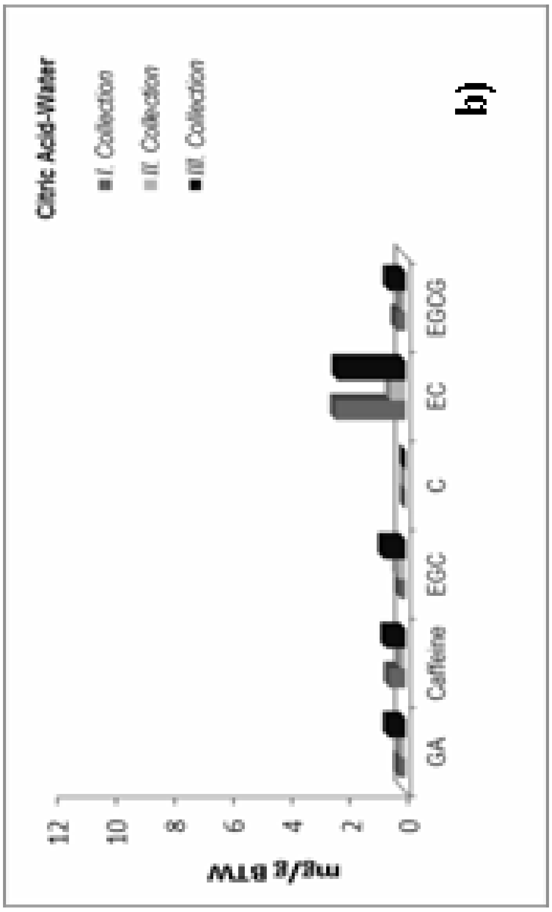
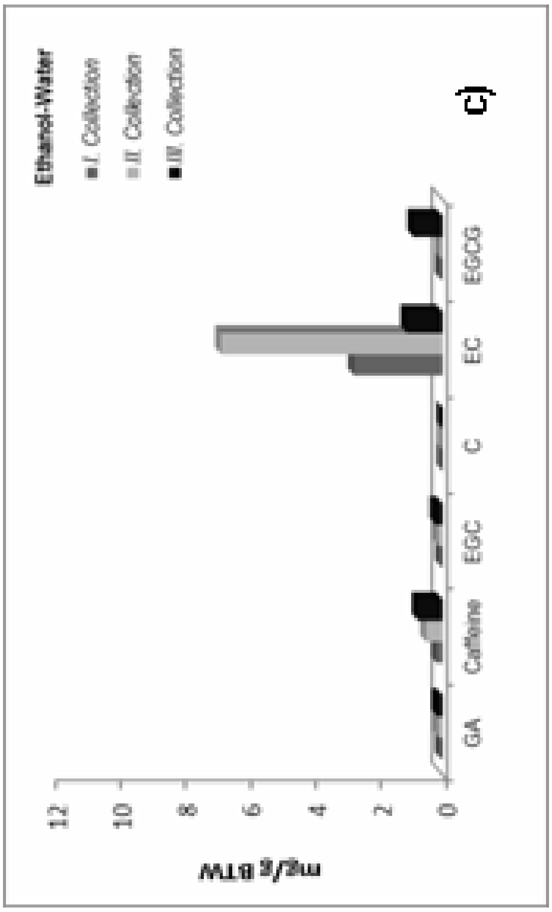
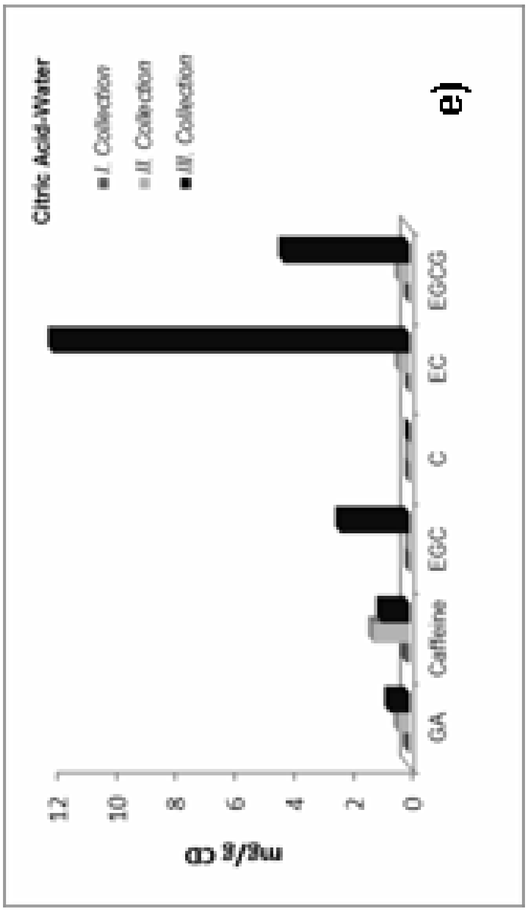


Figure 4. HPLC chromatogram of the catechin standard mixture. Gallic acid and caffeine were included into the standard mixture. Retention times are as following; gallic acid (GA): 7.00; caffeine: 11.03; epigallocatechin (EGC): 13.44; catechin (C): 16.05; epicatechin (EC): 20.44; epigallocatechin gallate (EGCG): 22.79 min. (λ=280 nm, injection volume: 20 μL).

The identification of catechins was carried out by comparing their retention times to standards and the amount of individual catechin was calculated from these chromatograms. The amounts of individual catechins present in the extract might give a good idea to compare extraction efficiency. Therefore quantified catechins from the HPLC chromatograms, along with gallic acid and caffeine residue, in the extracts obtained by MAE are given in Figure 5.


Citric Acid-Water
■ I. Collection
■ II. Collection
■ III. Collection
b)
mg/g BTW
0
2
4
6
8
10
12
GA
Caffeine
EGC
C
EC
EGCG


Ethanol-Water
■ I. Collection
■ II. Collection
■ III. Collection
c)
mg/g BTW
0
2
4
6
8
10
12
GA
Caffeine
EGC
C
EC
EGCG


Citric Acid-Water
■ I. Collection
■ II. Collection
■ III. Collection
e)
mg/g CD
0
2
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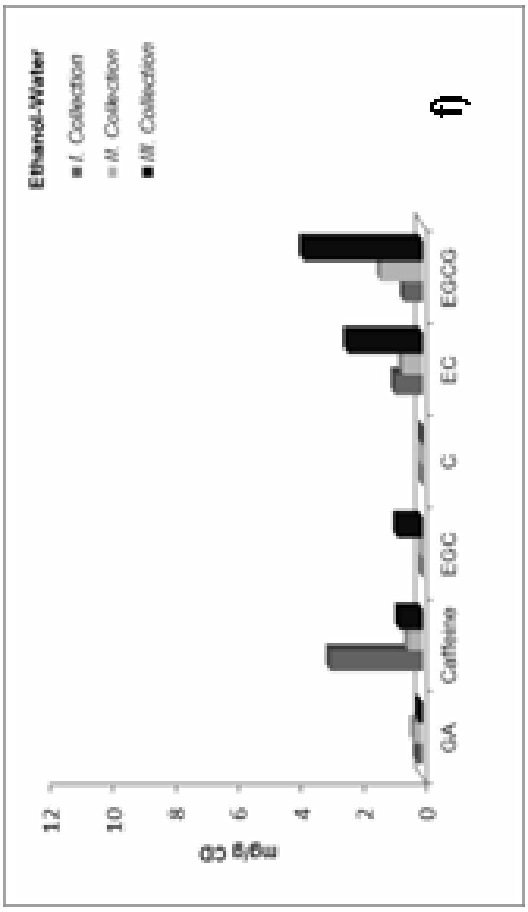
Caffeine
EGC
C
EC
EGCG


Ethanol-Water
■ I. Collection
■ II. Collection
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EGC
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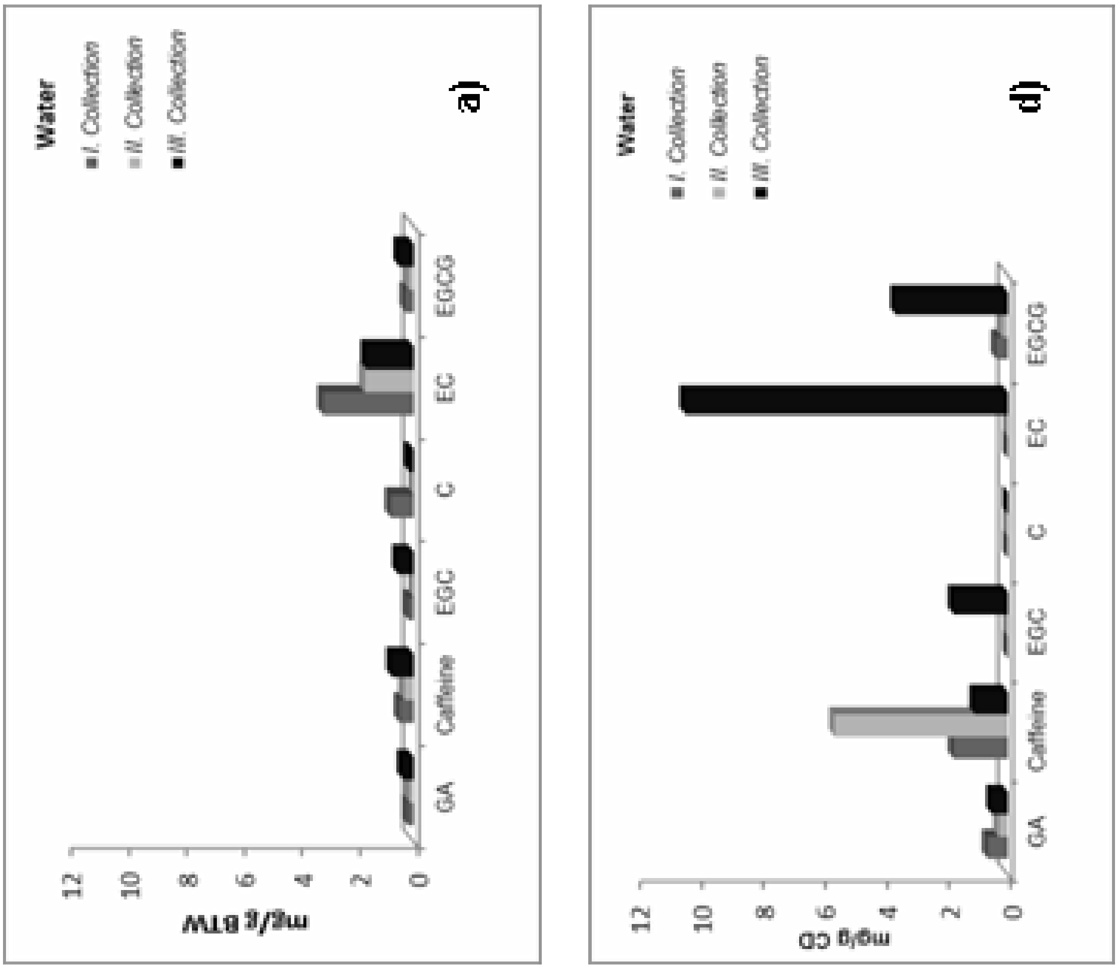
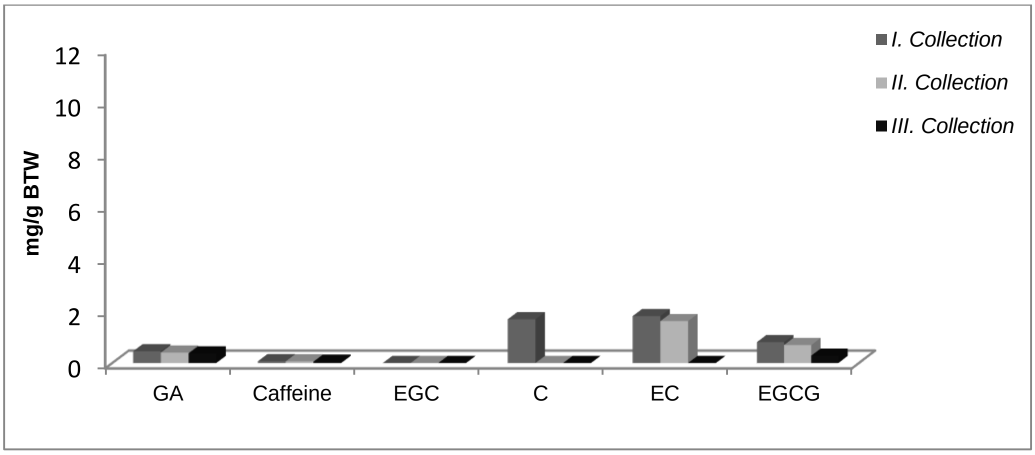


Figure 5. Quantification of catechins present in microwave extraction of BTW (a-c) and CD (d-f).

Quantification studies provided good information about which extraction solvent was more fruitful for effective extraction of individual catechins. As seen from Figure 5a-c BTW contains lower catechins than CD samples (Figure 5d-f). Concentration of EC is higher than other catechins in both waste materials but CD has also higher amount of other catechins such as EGC and EGCG. Extracted catechins were significantly high in the third collection period. The amounts of extracted catechins, especially EC and EGCG, are quite high in MAE in water and citric acid-water mediated systems. Citric acid must be providing an acidic medium for successful separation of EC and EGCG. It should be noted that citric acid-water solvent system extracts less caffeine than water mediated MAE. Therefore, it seems to be more selective to extraction of catechins. The amount of catechins obtained form conventional hot water extraction is given in Figure. 6.

a)



b)

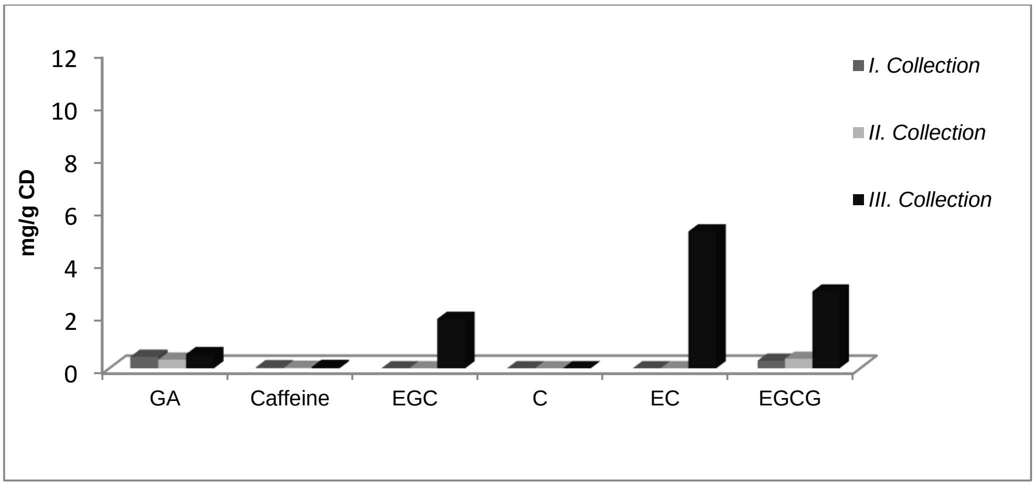


Figure 6. Quantification of catechins present in conventional hot water extract of **a)** BTW and **b)** CD.

Hot water extraction provided lower catechin constituent than MAE. Extract obtained from BTW has lower EC (ranged between 0.01-1.80 mg/g) and EGCG (0.28-0.82 mg/g) in all collection periods (Figure 6a). In CD extracts, EC concentration was extremely low in the first two collection periods. It increased to 5.21 mg/g in the third collection but still nearly half of MAE of the same sample (Figure 6b).

It is clear that extract yields are not solid evidence how extraction is successful for effective separation of these valuable chemicals. This study provides useful information related to effective extraction of tea waste employing microwave power. Separation of catechins was achieved in shorter extraction period (4 minutes), proposed MAE provide high extract yields and it is selective to EC and EGCG. MAE can be used as an effective, economical and time saving technique to obtain caffeine and catechins from tea wastes such as BTW and CD. Using citric acid-water for extraction in MAE enhanced the EC and EGCG constituent and might be used for selective extraction of these compounds.

The waste materials used in this study contain quite high amount of caffeine and remarkable amount of catechins. As a result, tea waste produced in Black Sea region can be considered as valuable bio-resource and MAE at large scale might be favored for energy cost industrial applications.

Acknowledgements

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Conflict of interest

This study does not have any conflict of interest.

4. References

[1]. Serpen, A., Pelvan, E., Alaşalvar, C., Mogol, B.A., Yavuz, H.T., Gökmen, V., Özcan, N. & Özçelik, B. (2012). Nutritional and Functional Characteristics of Seven Grades of Black Tea Produced in Turkey. *Journal Agriculturel of Food Chemistry*, 60 (31), p. 7682–7689

[2]. Pan, X., Niu, G. & Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing*, 42, p. 129-133.

[3]. Wang, L., Qin, P. & Hu, Y. (2010). Study on the microwave-assisted extraction of polyphenols from tea. *Frontiers of Chemical Engineering in China*, 4(3): 307–313.

[4]. Vuong, Q.V., Golding, J.B., Nguyen, M. & Roach, P.D. (2010). Extraction and isolation of catechins from tea. *Journal separation of science*, 33(21) p. 3415-3428.

[5]. Vuong, Q.V, Stathopoulos, C.E., Nguyen, M.H., Golding, J.B. & Roach, P.D. (2011). Isolation of Green Tea Catechins and Their Utilization in the Food Industry. *Taylor & Francis*, 27(3), p.227-24.

[6]. Row, K.H. & Jin, Y. (2006). Recovery of Catechin Compounds From Korean Tea by Solvent Extraction. *Bioresource Technology*, 97, p. 790-793.

[7]. Karadeniz, B. & Koca, İ. (2009). Phenolic Profiles and Antioxidant Properties of Turkish Black Tea Manufactured with Orthodox Method. *Asian Journal of Chemistry*, 21, p. 6803-6810.

[8]. Bronner, W.E. & Beecher, G.R. (1998). Method for determining the content of catechins in tea infusions by high-performance liquid chromatograp. *Journal of Chromatography A*, 805, p. 137-142

[9]. Wang, K., Liu, Z., Huang, J., Fu, D., Liu, F., Gong, Y. & Wu, X. (2009). TLC seperation of catechins and theaflavins on polyamide plates. *Journal of Planar Chromatography*, 22(2), p. 97-100.

[10]. Vovk, I., Simonovska, B. &Vuorela, H. (2005). Separation of Eight Selected Flavan-3-ols on Cellulose Thin-Layer Chromatographic Plates. *Journal of Chromatography A*, 1077, p. 188-194.

[11]. Goodarznia, I. & Govar, A.A. (2009). Superheated Water Extraction of Catechins from Green Tea Leaves: Modeling and Simulation Transactions C. *Chemistry and Chemical Engineering*, 16(2), p. 99-107.

[12]. Gürü, M. & İçen, H. (2004). Obtaining of caffeine from Turkish tea fiber and stalk wastes. *Bioresource Technology*, 94, p. 17–19.

[13]. Spigno, G. & De Faveri, D.M. (2009). Microwave-assisted extraction of tea phenols: a phenomenological study. Journal Food Engineering, 93, p. 210–217.

[14]. Deng, C.H., Ji, J., Li, N., Yu, Y.J., Duan, G.L., & Zhang, X.M. (2006). Fast determination of curcumol, curdione and germacrone in three species of curcuma rhizomes by microwave assisted extraction followed by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Journal Chromatography A*, 1117, p.115-120.

[15]. Nkhili, E., Tomao, V., Hajji, H.E., Boustani, E.S.E., Chemat, F. & Dangles, O. (2009). Microwave-assisted water extraction of green tea polyphenols. *Phytochemical Analysis*, 20(5), p. 408-415.

[16]. Wang, K., Liu, F., Liu, Z., Huang, J., Xu, Z., Li, Y., Chen, J., Gong, Y. & Yang, X. (2011). Comparison of catechins and volatile compounds among different types of tea using high performance liquid chromatograph and gas chromatograph mass spectrometer. *International Journal of Food Science and Technology*, 46 (7), p. 1406-1412.

[17]. İçen, H. & Gürü, M. (2009). Extraction of caffeine from tea stalk and fiber wastes using supercritical carbon dioxide. Journal Supercritical Fluids, 50 (3), p. 225–228.

[18]. İçen, H. & Gürü, M. (2010). Effect of ethanol content on supercritical carbon dioxide extraction of caffeine from tea stalk and fiber wastes. *Journal Supercritical Fluids*, 55 (1), p. 156-160.

[19]. Shalmashi, A., Abedi, M., Golmohammad, F. & Eikani, M.H. (2010). Isolation of caffeine from tea waste using subcritical water extraction. *Journal of Food Process Engineering*, 33 (4), p. 701-711.

[20]. Oliveira, M.E.C., & Franca, A.S. (2002). Microwave heating of foodstuffs. *Journal of Food Engineering*, 53 (4), p. 347-359.