Journal of Medicinal Plants and By-products (2019) 1: 77-83

Original Article

Investigation of Consecutive Separating Arrangements of Bio active Compounds from Black Tea (*Camellia sinensis*) Residue

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Article History: Received: 01 September 2018 /Accepted in revised form: 19 November 2018

Abstract

Every year lots of black tea (*Camellia sinensis* (L.) Kuntze) residue will produce in the factories. These residue are unusable whereas the bio active compounds can be extracted and used in the drag and food industries. Due to mentioned problems, this project was conducted years 2011 - 2012 with the aim to make a study on consecutive isolation of all bio active compounds from tea residue, that extraction of one compound won't benefite to build a lateral products factory but isolation of all bio active compounds can increase productivity. In this survey, four compounds of caffeine, catechin, fiber and protein were be separated and measured from residue mixture in three steps from three sequential models. The isolation of caffeine and catechin were placed together in one step because the extraction condition was similar. Experiments was conducted with four replications and data was analyzed. The results indicated that effect of three sequence models was significant on extraction yield of caffeine, catechin, protein, and fiber (P<0/01). Comparison of yields indicated that the maximum amount of caffeine and protein was obtained from second sequence, also the maximum amount of catechin and fiber from third sequence. The economic comparison results among sequences indicated that the all sequences were economical however third sequence was introduced as the most economical model in terms ratio of benefit to cost due to high price of catechin and maximum rate

Keywords: Black tea (*Camellia sinensis*) residue, Consecutive separating, Bio active compounds

Introduction

Tea is known as *Camellia sinensis* (L.) O. is the species of plant whose leaves and buds are used to produce tea. It is of the genus *Camellia*, a genus of flowering plants in the family *Theaceae* that contains nearly 4000 of bio active compounds of which one third is contributed by polyphenols [1, 2]. The other compounds are alkaloids, aminoacids, carbohydrates, proteins, chlorophyll, volatile organic compounds, fluoride, aluminum, minerals and trace elements. Polyphenols found in tea are mostly flavonoids [3, 4]. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits that have traditionally been attributed to tea, especially green tea [5]. Major catechins are epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate. the most active and abundant catechin in tea is epigallocatechin gallate, that has been shown a significant role as antioxidant, antibacterial, antiviral, lipid lowering, hypoglycemic, hypotensive and DNA hypermethylation inhibition, telomerase inhibition, metastasis inhibition, caspase inhibition and metalloproteinase inhibition [6].

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Caffeine is a bio active compound of tea and some products such as coffe, guarana and cacao. They usually taste bitter and often are physiologically active in human and acts as stimulants of the central nervous, muscle and circular systems and consumed in pharmaceuticals and beverages. It creates health risks in children, pregnant and some patient [7].

Protein of tea has a wide variety of biological functions in many aspects such as antitumor, antiinflammation, antivirus, lowering blood sugar, anticaducity and anticoagulant [8, 9].

Fiber is a group of tea polysaccarids that used in the food industry and in medicine for a long time due to biological activities. It is one of the main bio active components of tea that has activities of immunologic, antioxidant, antiradiation, anticoagulation, anticancer, anti-HIV and hypoglycemic [10].

The sequential extraction of the important compounds of residue was reported first time by Wicramassinge. Caffeine (1.5-3.5%), pigment of polyphenols (8-10%), protein (10-15%), and fiber (15-20%) were extracted respectively by sequential model [11] also residue of tea factories was studied as edible color [12]. Tea stalk and fiber residues have no economical value so cheap raw material makes investigation of extraction of bio active compounds from tea residue a promising program for technological applications which it is neccessery [7].

The purpose of this project was investigative of sequences arrangments economically to isolate all important pharmaceutical compounds from Iranian tea residue.

Material and Methods

The mixture of Iranian black tea fluff, stalk and fiber residues was employed in this study was provided by five tea factories in the Lahijan city. The samples of tea residue were blended and then dried at 105±2 °C for 2 h. these residues were ground using a cutting mill and sieved. Four compounds of caffeine, catechin, protein and fiber were extracted in four replicates. Caffeine, catechin, protein and fiber was named respectively A, B, C and D (Table 1). Caffeine and catechin were grouped together because they are strongly dissolved in water. The molecular mass of caffeine, catechin, protein and fiber is 194.19, 424.724, 1701.20, and 20,000 g/mol respectively.

Table 1 Consecutive separating arrangements

Sequences	Step1	Step2	Step3
First Seq.	A and B	C	D
Second Seq.	C	A and B	D
Third Seq.	D	A and B	C

Data were analyzed with the statistical design completely randomized. Analysis and comparsion of data was performed respectively by SAS and LSD.

Outline of Orocess

First Sequence

Tea residue was refluxed for 1.5 hours then A and B compounds were extracted by solvents (Step 1). Residual was refluxed with alkaline solution for 2 hours and C compound was precipitated from extract by acidic reagent (Step 2). Residual of second step was refluxed once with acid and once with alkaline for 1 hour and D compound as residual was obtained (Step 3).

Second Sequence

Tea residue was refluxed by alkaline solution for 2 hours and C compound was precipitated from extract by acidic reagent (Step 1). Compounds of A and B were extracted from extract rest of first step by solvents (Step 2). Residual was refluxed once with acid and once with alkaline for 1 hour and D compound collected as residual (Step 3).

Third Sequence

Tea residue was refluxed for one hour once with acid and once with alkaline and D compound as residual was obtained (Step 1). A and B compounds were extracted from acidic extract of first step by solvents (Step 2). C compound was precipitated from alkaline extrac of first step by acidic reagent (Step 3).

Determination of Components Content

Caffeine: ten grams of tea residue was refluxed in 200ml water for 30 minutes at 100 °C and this work was repeated three times and extract was filtered, this section was conducted only in the first sequence. In the second and third sequences, the rest of first step extract was used. In the following, caffeine was extracted from extract in four parts with 200 ml chloroform by separatory funnel after separating of two phase, solvent was evaporated and white crystals of caffeine was collected,

weighted and determined their melting point 238 °C (the caffeine melting point reported in the literature is 238 °C). Standard caffeine solution was analysed at different concentrations by spectrophotometer and the results were used as calibration graph (figure1). Caffeine content was determined at 272 nm wavelenghth [13].

Catechin: the equal volume of ethyl acetate was added to the water phase of the separator funnel from the previous section of caffeine extraction. This extraction was repeated three times and solvent was evaporated, the residual was the bright brown catechin powder [14].

Standard of gallic acid is used to determine catechin. Different concentrations of gallic acid solution was analysed by spectrophotometer and the results were used as calibration graph (Fig. 2). catechin content was determined at 765 nm wavelenghth [15].

Protein: ten grams of tea residue and residual in the second and first sequences respectively was refluxed at 0.08 M sodium hydroxid in the constant volume-weight ratio of 35:1 for 2 hours and the constant temperature of 30 °C then it was filtered and added the chloridric acid to extract to neutralize and solution was placed in the environment to precipitate and was filtered [16], amount of protein was quantified using the Kjeldahl method [17]. In the third sequence, protein content was determined from alkaline extract rest of first step.

Fiber: ten grams of tea residue in third sequence and residuals in the first and second sequences were refluxed in 0.255 M sulfuric acid solution for 30 min, the insoluble residue was filtered and washed. The obtained substance was subsequently refluxed in 0.313 M sodium hydroxide solution, filtered and washed. The residual was collected as fiber [18].

Results and Discussion

Compounds extraction yield from plant depends highly on the extraction condition such as agent concentration (pH value of the solution, enzyme), extraction temperature, extraction time, volume wight ratio between the extraction solvent and the raw material [19, 20]. In this study, NaOH and H_2SO_4 was used alkalin and acidic agents. This research demonstrated consecutive separating arrangements due to be different extraction conditions is significant on the yield of caffeine, catechin, protein and fiber at 1% level. The results of analysis of variance were brought in table 2. The partial budgeting approach was used for the economic comparison among sequences [21]. Investigate ratio of income to cost for three sequences were brought in table 7. The result indicated that third sequence is the best sequernce in terms of the income to the cost.

Comparison of Sequences

Caffeine

Caffeine is 1,3,7-trimethylxanthine a member of organic compounds call alkaloids. Caffeine content is by 1.5 to 3% in tea residue and 2-4% in tea. In the industries, maximum amount of caffeine was extracted from the alkalin solution of 0.1 M sodium hydroxide due to low alkaline properties of caffeine. It is one of the most consumed dietary ingredients in the world. Tea, its residue and coffee are the main prominent sources [22-27].

The comparison results of sequences showed that the maximum caffeine yield was obtained 1.432% from the second sequence due to alkaline solution of first step at protein separation and ranked first (table 3). In the first sequence, extraction was conducted in water and condition was ordinary and extraction yield was obtained 0.9815% and ranked second. in the third sequence, the extraction was done with a lower yield 0.9669% due to acidic solution of first step at fiber separating and ranked third.

Catechin

Catechin belong to those groups of compounds as flavonoids. The maximum amount of catechin in the black tea 4% and in green tea 14% is reported [28- 30]. Catechin is used in various industries such as drag, food, cosmetics [14]. it has been reported that extraction yield of catechin was increased in pH 2-4 due to acidic properties of catechin [31], also the extraction of natural compounds increased in alkaline solutions less than 0.2 N sodium hydroxide due to the destruction of the cell wall and the release of compounds from cell but in sodium hydroxide more than 0.2 N, the extraction yield was decreased because natural compounds were decomposed [32].

The comparison results of sequences showed that maximum catechin yield was obtained 1.328% from third sequence due to the acidic solution of first step at fiber separating and also maximum rate and income and ranked first (table 4). In second

sequence, the catechin was extracted after of protein separation by sodium hydroxide 0.08 N and extraction yield was obtained 1.124% and ranked second. In the first sequence, catechin extraction was conducted in water and condition was ordinary and extraction yield was obtained 0.9301% and ranked third.

Fiber

Fiber is a simple suger. Tea leaves consist mostly of fiber, a water- insoluble polymer of glucose, It is structure building material. Amount of fiber in black tea residue 5-43% is reported [22,26,33] along with fiber are found a number of other things including caffeine, tannins or phenolic compounds that have an OH directly boned to an aromatic ring and a small amount of chlorophyll. Tea fiber can be utilized in the industries of pharmaceutical, food, paper and building materials [10].

The comparison results of sequences showed that the maximum fiber yield was obtained 38.922% to standard method from the third sequence and also maximum rate and income and ranked first (table 5). In the first and second sequences, the fiber separation was conducted in the third step, fiber yield was decreased to 35.026% and 36.152% and ranked third and second respectively. The fiber yield in acidic and alkaline concentrations was decreased due to further exiting of soluble compounds.

Protein

Researches show that protein content is 21-28% in tea leaves and 11-15% in tea residue on dry base. The nutritional value of tea residue is related to the protein content that can be used in the food industry as a foaming agent or as functional ingredients for nutrient delivery, also in cosmetic products. It was recently reported that tea protein has the capability in protecting biological cells against mutagenesis caused by irradiation. The use of alkaline solutions has been widely recognized as a feasible method for protein separating from plant sources [16, 22, 26].

The comparison results of sequences showed that the maximum protein yield was obtained 15.25% from the second sequence and ranked first (table 6). In the first and third sequences, protein yield was decreased to 8.772% and 13.41% respectively due to acidic condition, higher temperature, lower extraction time and lower volume-weight ratio at the beginning of sequences and ranked the third and second respectively.

Economic Analysis

The partial budgeting approach was used to obtain the best sequence in terms of economical productivity [21]. The cost of buying chemicals and gross income for 100 grams of residue for three sequences are shown in table 7. The treatments were compared to choose the best treatment in terms of the income to the cost. The third sequences were introduced as the best economical sequences with the maximum income to cost ratio of 7.9257.

Table 2 Results of variance analysis

Sources of change	Degrees of freedom	average of squares			
		caffeine	catechin	fiber	protein
Treatment	2	0.659^{**}	0.314^{**}	26.738^{**}	34.11^{**}
Error	9	5.555×10^{-5}	0.033	0.603	0.002
c.v.		0.67	19.60	2.14	1.30

Table 3 Results of percentage, efficiency, measurement rate and gross income of caffeine

sequences	percentage	Efficiency ⚐	Measurement rate@	Gross income in rials §
2	1.432	100	6.14×10^{-5}	222246.4
1	0.9815	68.5	5.61×10^{-5}	152320
3	0.9669	67.52	8.29×10^{-5}	150060
-	LSD (%5)	-	-	-

⚐ 100 × Experimental / Measured

@ Extract time to min / mol- 5 gr was $ 40.19 from sigma company.

Table 4 Results of percentage, efficiency, measurement rate and gross income of catechin

sequences	percentage	Efficiency ⚐	Measurement rate @	Gross income in rials§
3	1.328	100	5.21×10^{-5}	1851760
2	1.124	84	2.20×10^{-5}	1180200
1	0.9301	70	2.43×10^{-5}	976500
	LSD (%5)			

105 mg was 35$ from sigma company.

Table 5 Results of percentage, efficiency, measurement rate and gross income of fiber

sequences	percentage	Efficiency ⚐	Measurement rate@	Gross income in rials§
3	38.922	100	7.620×10^{-4}	29470
2	36.152	91	1.77×10^{-4}	27370
1	35.026	88	8.57×10^{-5}	26520
	LSD (%5)			

410 gr was 10.35 $ from sigma company.

Table 6 Results of percentage, efficiency, measurement rate and gross income of protein

sequences	percentage	Efficiency ⚐	Measurement rate@	Gross income in rials §
2	15.25	100	6.35×10^{-6}	187498
3	13.41	87	1.11×10^{-5}	173659
1	8.772	57	2.08×10^{-6}	113597
	LSD (%5)			

50 gr was 21.59 $ from sigma company.

Table 7 Investigate ratio of income to cost for three sequences

Treatsment	Sequences		
	First Sequence	Second Sequence	Third Sequence
Total cost of compounds	280600	280600	280600
Gross income	1268930	1617300	2204940
Income / cost	4.522	5.764	7.9257

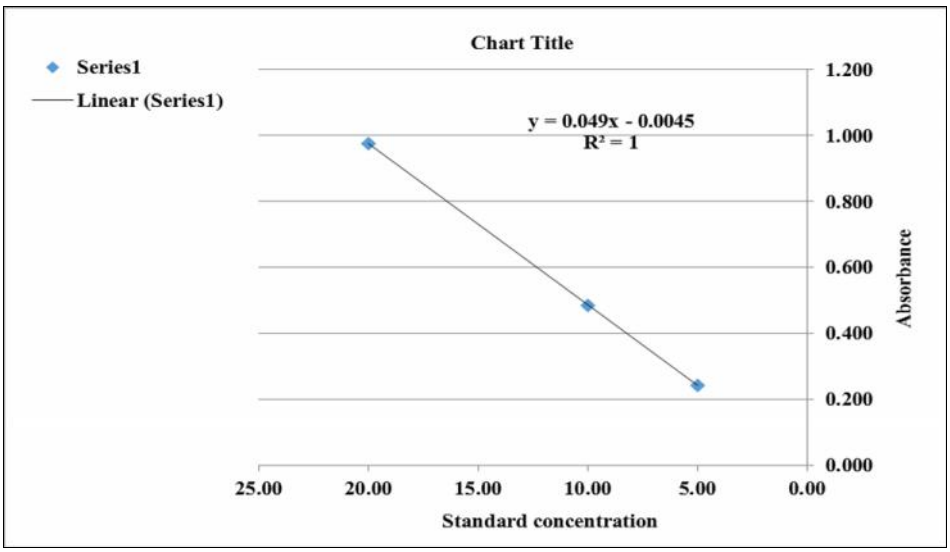

Fig. 1 calibration curve of caffeine standard

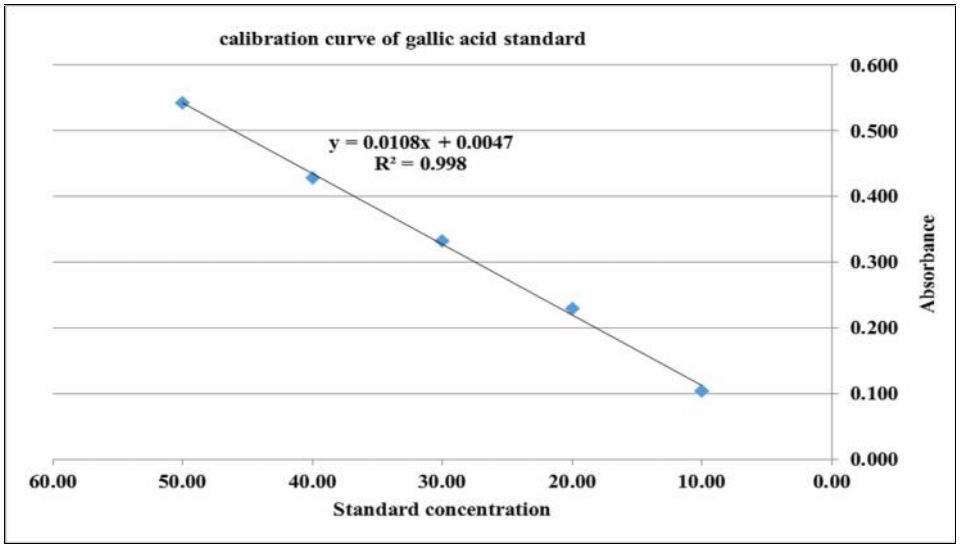

Fig. 2 calibration curve of gallic acid standard

Conclusion

In this study, it was found that, extraction conditions have a significant influence on components yield. The catechin yield at acidic concentration (pH 2-4) and the caffeine and protein yields at weak alkaline concentrations were increased and the fiber yield was decreased both in acidic and alkaline concentrations.

The economic comparison results indicated that the all sequences were economical however third sequence was introduced as the most economical model because it had the maximum income to cost ratio of 7.9257 compared to the second and first sequences 5.764 and 4.522 respectively due to the high yield and price of catechin (Table 7), also third sequence had maximum rate because separation lasted lower than other sequences.

Acknowledgements

We are grateful to acknowledge Tea Research Center, Agricultural Research, Education and Extension Organization, Lahijan, Iran for the financial support of this investigation.

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