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DETERMINATION SOME COMPONENTS IN DIFFERENT KINDS OF TEA: CAFFEINE, CATECHINS, GALLIC ACID AND THEOBROMINE[1]

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ABSTRACT

A high performance liquid chromatography method with reversed phase separation was performed on (C18) column for the separation of the high and weak polar components of local tea. The method was developed by the combination of isocratic and gradient elution methods. The types of teas which were used for determination and quantification of three catechins, alkaloids (caffeine, theobromine) and gallic acid were black tea, white tea, green tea, echo olong tea, yellow tea, Rooibos tea and Assam tea with different amount of the components . Theophylline contents are negligible because it has low concentration. The range of the calibration curves was from 10-600 µg/ml depending on which analytes that had been used. The totally run time was 35 min. The total amount of catechins was from 0.3-39 mg/g, the level of caffeine was 17-20 mg/g and gallic acid from 0.3-2mg/g tea.

Keywords: *Tea analysis, caffeine , Catechins, gallic acid, Camellia sinensis*

INTRODUCTION

Tea (*Camellia sinensis*) is one of the most popular beverages in the world. It comes from China from the beginning and produces in Southeast Asia and central Africa. Commercial teas can generally be classified into three major categories: nonfermented green teas, partially fermented oolong teas and fully fermented black and red teas. The difference between green, yellow, white, oolong and black tea are that they have different processing. White tea contains most catechins followed by green and black tea [1]. Yellow teas manufacturing look like green tea but the drying process is longer than that for green tea. Tea extracts has showed to have antioxidant, antimutagenic properties [2, 3].

Assam tea is a black tea and it got this name from the region of growing in India. It grows in large amount in southern china as well. It has many benefits which include boosting cardiovascular health, preventing some types of cancer, improving cognitive function, and speeding the metabolism [4]. Red tea does not come from *Camellia sinensis.* It comes from *Aspalathus linearis* and calls often Rooibos Tea or Red tea and comes from South Africa. Roobios tea has no or very low amount of caffeine in it and

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it has some antimutagenic effects. It is rather to be called as herbal tea than normal tea [5]. Catechins (**fig 1**) are polyphenolic antioxidants from plant metabolite and have showed to have antioxidant, antimutagenic properties [3].

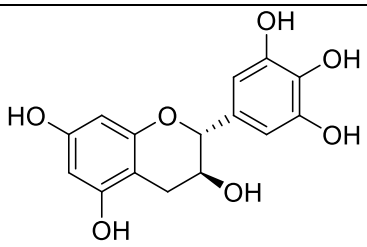
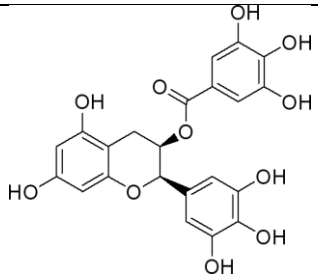
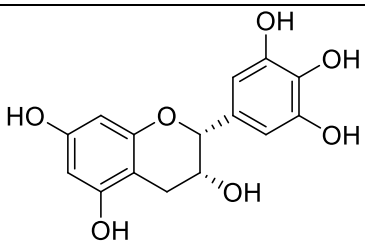
		
Catechin (C)	Epigallocatechin gallate (EGCG)	Epicatechin (EC)
Mp=240 °C	Mp=218 °C	Mp= 251 °C

Figure 1. Structure of Catechins.

Caffeine, Theobromine and Theophylline are xanthine alkaloids (**fig 2**). Caffeine can be found in many kinds of food for example coffee, chocolate and tea. Caffeine has effect on sleep, metabolism and also on mood [6]. Theobromine can be found in a high amount in chocolate and in tea as well. It is used in the treatment of angina pectoris and hypertension and it is a good diuretic substance [7]. Theophylline could be found in tea but in a very low amount. The biological effects of theophylline is cardiac stimulant [8]. Gallic acid (**fig 2**) is a food component that found especially in tea. It has showed to have antioxidant, antiinflammatoric, anticancerogenic effect [9].

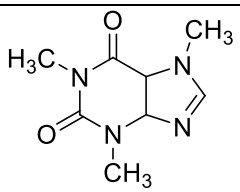
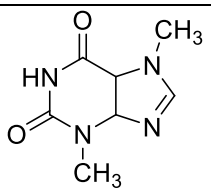
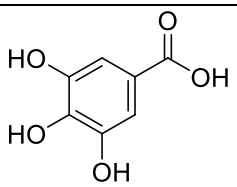
		
Caffeine (CAF)	Theobromine	Gallic Acid (GA)
Mp=234-236.5 °C	Mp=345-350 °C	Mp= 252 °C

Figure 2. Caffeine, Theobromine and Gallic Acid

MATERIAL AND METHODS

Chemicals and tea samples

Acetonitrile (Sigma-Aldrich; HPLC Gradient), Formic Acid (Scharlau; Regent Grade), Ethyl Acetate (Sigma-Aldrich; HPLC Gradient), Methanol (Sigma-Aldrich; Analytical gradient) Caffeine (Merck; Regent Grade). Gallic Acid, Epicatechin gallate, Theophylline, Catechin, Epicatechin and theobromine are all from Sigma-Aldrich and they were been used in HPLC-gradient. The water was obtained by MilliQ plus system.

Different kinds of tea samples were collected from various local shops. We used the following teas in our project: Black tea (Lipton yellow label), white tea (Lipton), green tea double dragon, green "Eko olong", sunflower yellow tea "Rooibos vanilj tea" (Forsman tea) and Assam Mokalbari tea.

Instrumentation

The HPLC system was Agilent 1100 which was used to identify and quantify the analytes in different kinds of tea. The sample loop was 20 µL. The column was a Reprosil 80 ODS C18 column (4.6 mm id, and 5 µm particle diameter). Detection was carried out by using an UV detector which measured absorbance at 280 nm.

The mobile phase was composed of acetonitrile (solvent A) and 0.1% formic acid water solution (solvent B, v/v). The mobile phase was prepared by degassing it for 5 min in an ultrasonic bath. The mobile phase flow rate started with 0.7 ml/min for 16 min, and then increased linearly to 1.7 ml/min in 17 min. After 36 min, the flow decreased to 0.7 again.

Method

Stock solutions preparation

Stock solutions of catechin (631.8 µg/mL), epigallocatechin gallate (EGCG) (600 µg/mL), epicatechin (EC) (548.6 µg/mL), Caffeine (CF) (666 µg/mL), Gallic acid (540 µg/mL), Theophylline (592 µg/mL) and Theobromine (600.1 µg/mL) were prepared by dissolving the pure standard in the mobile phase . Low concentration solutions as needed for calibrations and determinations were prepared by diluting it in the same mobile phase. The range of the different analytes was (10-600 µg/ml) depending on the analyte [10].

Sample preparation

Tea samples were prepared by weighing approx 1 g of the tea dissolved in 50 ml of the mobile phase and extracted by ultrasonic for 5 min at room temperature. The solutions of the samples were filtered through 0,45 µm nonpyrogenic filter, then injected on the HPLC. The samples were run three times for each sample (one sample for each tea).

Table 1. The weight of tea samples, each sample run three times

Brand	Weight (g)	Volume (ml)
Lipton yellow label	1.1320	50
Lipton White tea	1.5130	50
Ekologist Oolong tea	1.0504	50
Sunflower yellow tea	1.1139	50
Rooibos vanilj tea	1.1512	50
Green tea	0.4220	50

RESULT

Standard solution

The standard solution mixture of Gallic Acid (GA) (540 µg/mL), Theobromine (Theo) (600.1 µg/mL), Theophyline (592 µg/mL), Caffeine (Caf) (666 µg/mL), Catechin(631.8 µg/mL), Epicatchin (Epi) (548.6 µg/mL) and Epigallocatcin gallate (ECGC) (600 µg/mL) was eluted and the retention time with peak areas of these components were determined (**fig 3**).

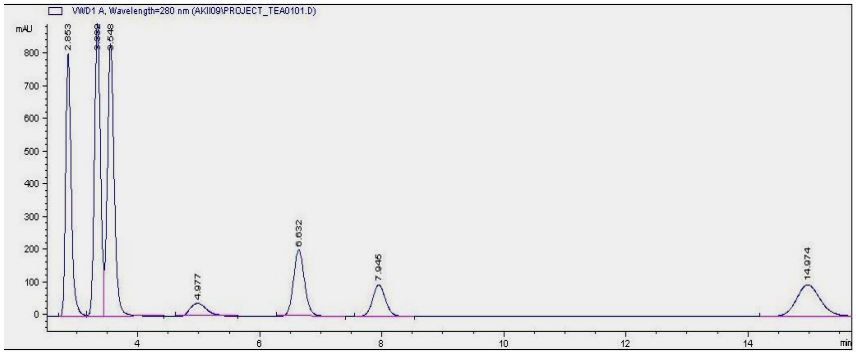


Figure 3. Chromatogram of Standard mixture, Gallic Acid (GA) (2.853); Theobromine (Theo)(3.338); Theophyline (3.548); Caffeine (Caf) (4.977); Catechin (6.632); Epicatchin (Epi) (7.945); Epigallocatcin gallate (ECGC)(14.974)

In (fig 3), we get the retention time of the standard solution of different compounds (table 2).

Table 2. The retention time of each standard solution component

Standard compound	Retention time
Gallic Acid (GA)	(2.853)
Theobromine (Theo)	(3.338)
Theophyline	(3.548)
Caffeine (Caf)	(4.977)
Catechin	(6.632)
Epicatchin (Epi)	(7.945)
Epigallocatcin gallate (ECGC)	(14.974)

Elution method

In order to get the optimum composition of the mobile phase and best procedure of separation, the isocratic, gradient and a combination of both elution methods were applied. The mobile phase composed of acetonitrile (solvent A) and 0.1 % formic acid in water (solvent B).

By applying the total isocratic method with 10% solvent A from the injection time until the end of the elution, the separation was not good for some components. Some of the peaks in chromatogram were not clear and they were overlapped due to the rapid elution (**fig 4**).

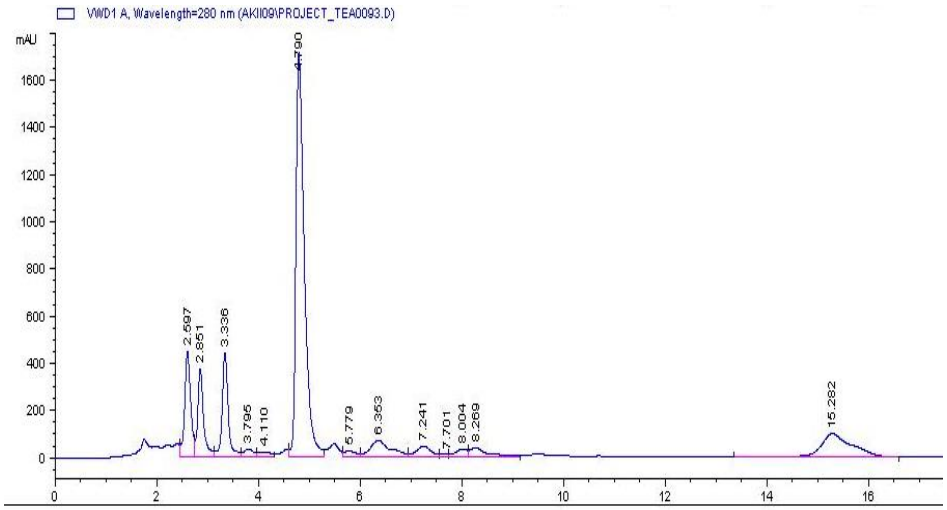


Figure 4. Yellow label on isocratic run (GA) (2.851); (Theo)(3.336); (Caf) (4.790); (Catechin) (6.353); (ECGC)(15.282)

The total gradient method of the sample was applied by eluting 10-50% solvent A for 20 min. The separation of the peaks was not achieved. In addition, the baseline was high and the overlapping of peaks was observed (**fig 5**).

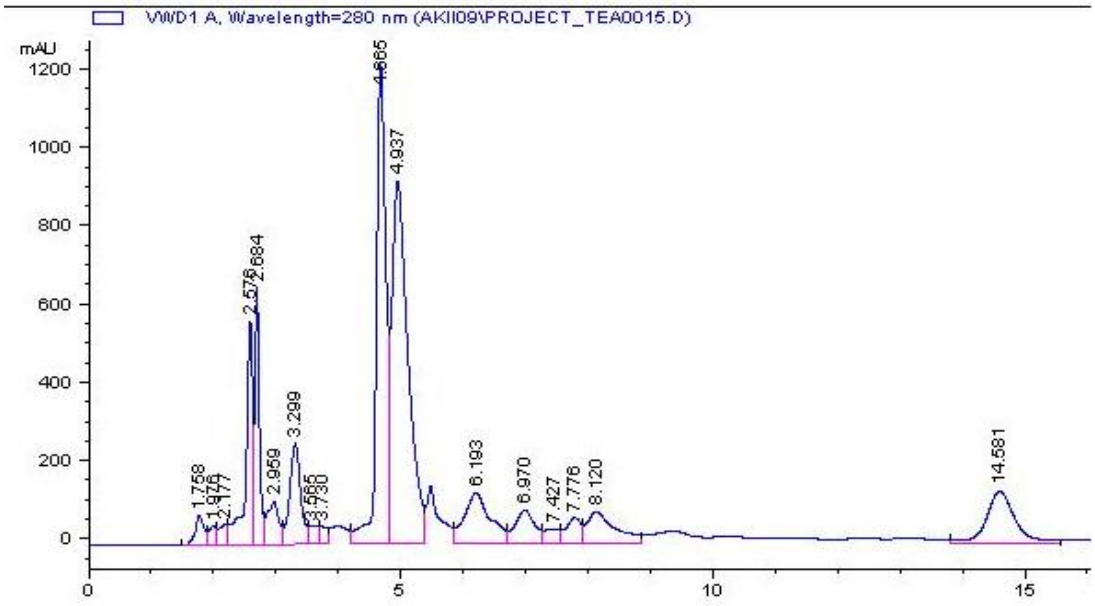


Figure 5. Yellow Label tea on total gradient run

The separation of first peaks like caffeine was achieved by using 6-10% solvent A mobile phase in the gradient elution with slow flow rate (0.7 ml/min for 16 min) (**fig 6**).[11, 12].

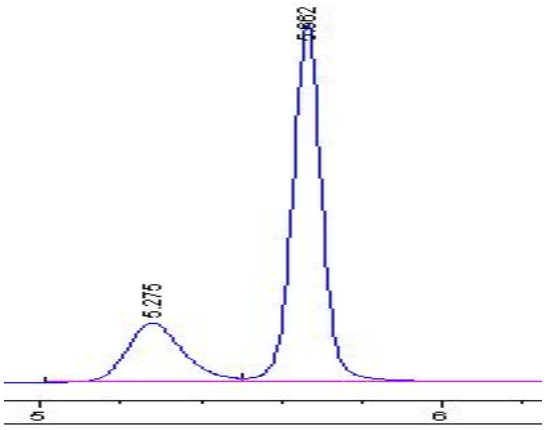


Figure 6. Gradient run of caffeine with water mobile phase

The combination of gradient and isocratic method was developed and applied on different samples. The optimal separation of the components of interest was achieved through a combination of isocratic and gradient elution methods using acetonitrile and a 0.1% formic acid in aqueous solution (v/v) for over 35 min. The mobile phase was composed of solvent A acetonitrile: solvent B water with methanol, ethyl acetate, glacial acetic.

The combination elution was achieved by starting gradient elution with 6% to 10% solvent A from the injection time until 10 min, then to isocratic 6% solvent A over the next 10 min, a further linear gradient from 12% to 20% A for 15 min.

It was started with a gradient method but changed to isocratic method to achieve best separation (**fig 7, 8, 9, 10**). The mobile phase flow rate started with 0.7 ml/min for 16 min, and then increased linearly to 1.7 ml/min in 17 min. After 36 min, the flow decreased to 0.7 again. The temperature of the column was 10 °C, and the injection volume was 10 µL.

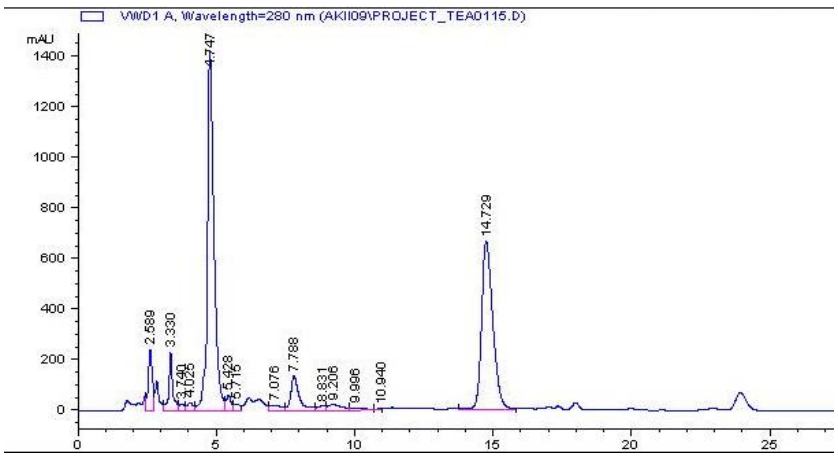


Figure 7. Green Tea; (Theo)(3.330); (Caf) (4.747); (Epi)7.788 ;(ECGC)(14.974)

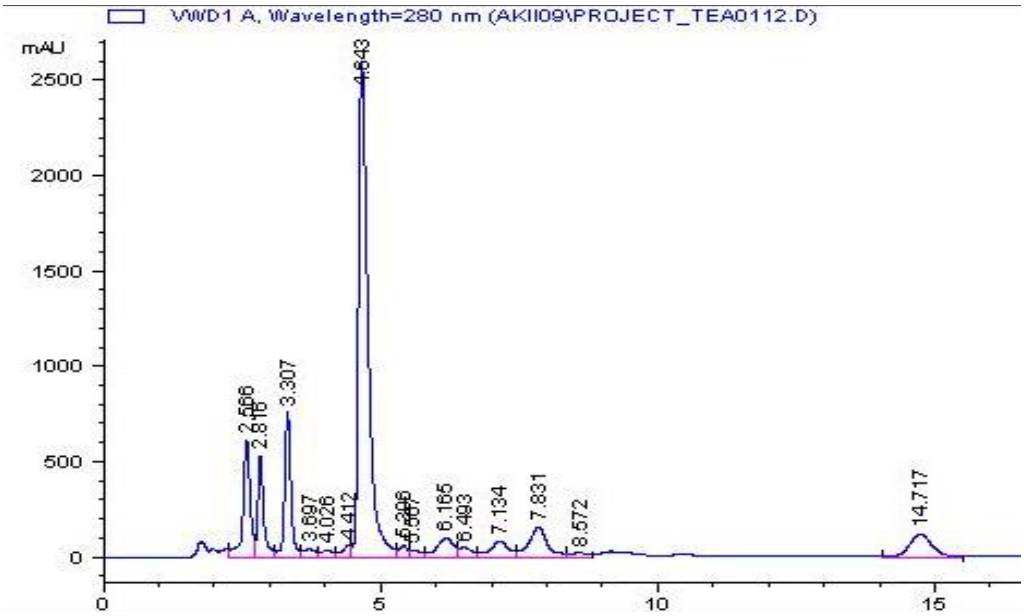


Figure 8. Assam Molakri tea; (GA) (2.816) (Theo)(3.307); (Caf) (4.643); (C) (6.165)(Epi)7.831 ;(ECGC)(14.974)

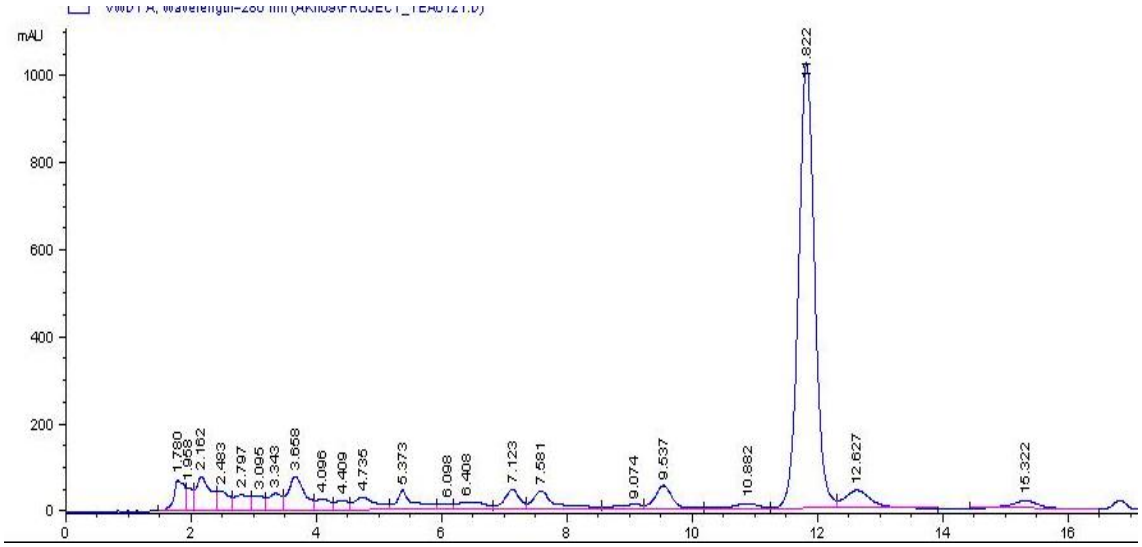


Figure 9. Roobios tea; (GA) (2.797)

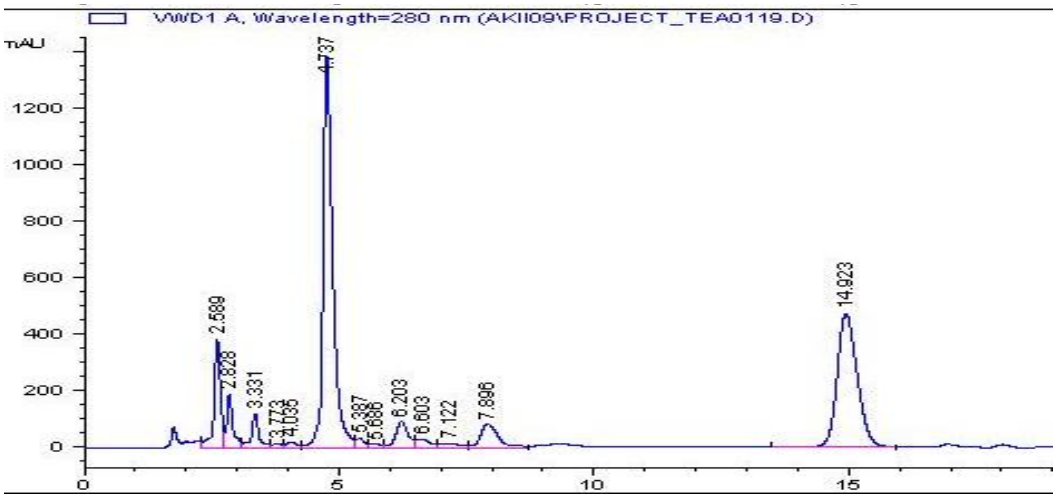
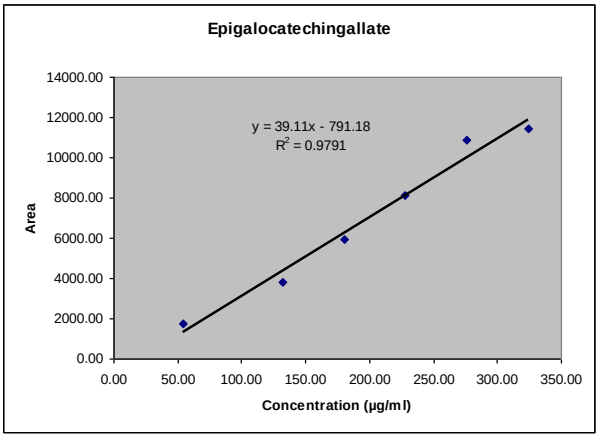
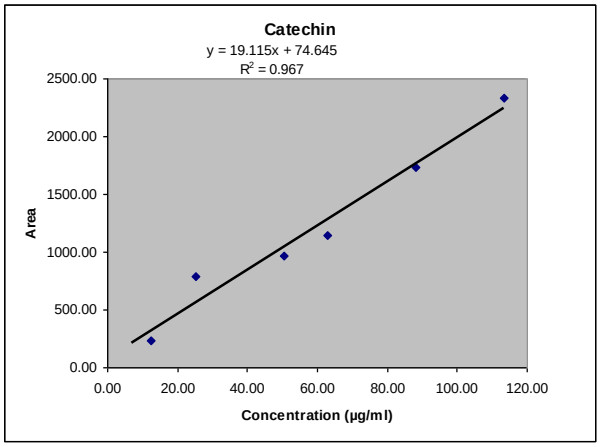


Figure 10. Yellow Sunflower; (GA) (2.828) (Theo)(3.331); (Caf) (4.737); (C) (6.203)(Epi)(7.896);(ECGC)(14.923)

Data analysis

The calibration curves of different components were obtained at wave length 280 nm [11]. The calibration curve between the area and concentration in (µg/ml) was applied for components in different tea samples and standards. The linearity plot of calibration curve was obtained with correlation coefficient between 0.998 for theobromine to 0.967 for catechin (**fig 11**).





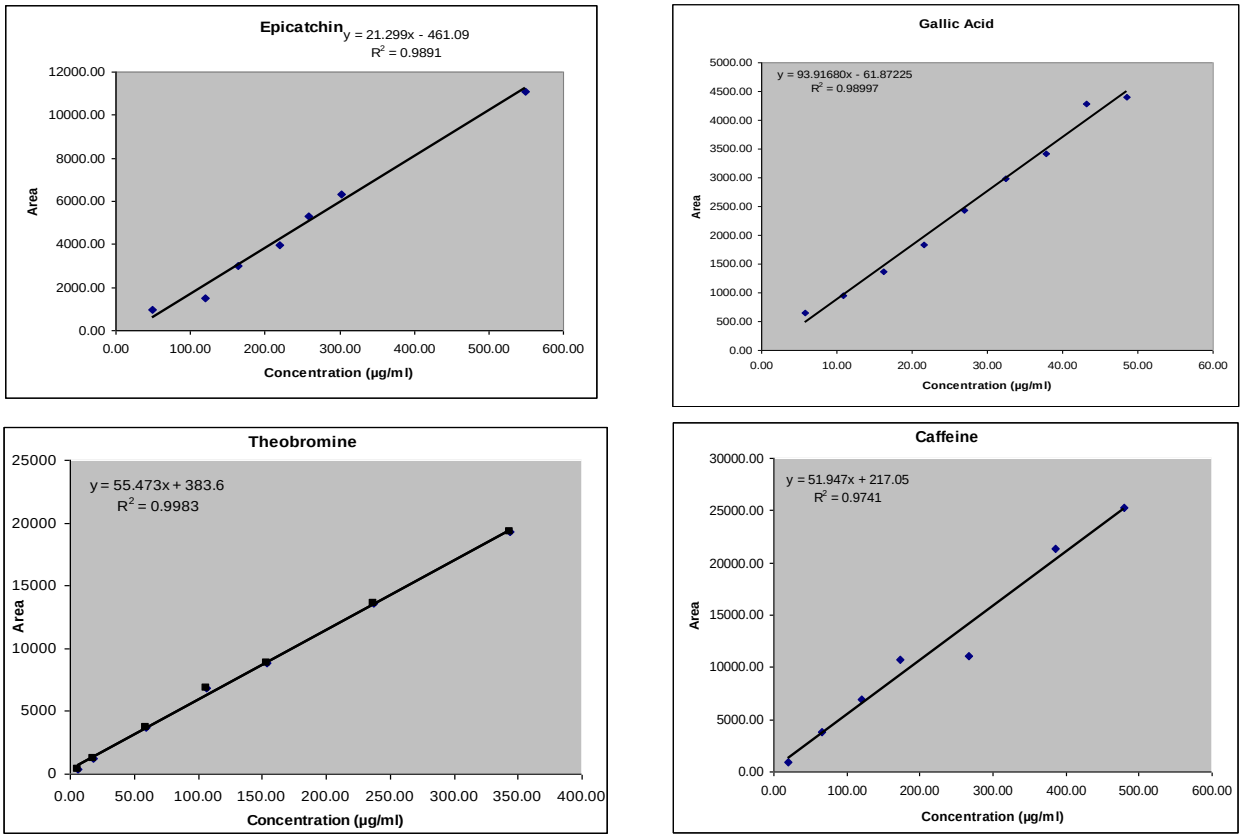


Figure 11. Linearity plots showing the correlation coefficient for catechin 0.967, caffeine 0.974, epicatechin 0.989, epigallocatechin gallate 0.979, theobromine 0.998 and gallic acid 0.990.

For each analyte, the concentration was compared with the area of the peaks in the chromatograms and chart. The samples were run three times in the same mobile phase and conditions. The average concentration in mg/g of the three run was calculated and the standard deviation of each component in the sample was determined (**table 3**).

Table 3. Results that we got in mg/g for samples run three times on each sample

Brand of tea	**Caffeine**	**Gallic Acid**	**Theobromine**	**Catechin**	**Epicatechin**	**Epigallocatechin gallate**
	Mean value SD mg/g	Mean value SD mg/g	Mean value SD mg/g	Mean value SD mg/g	Mean value SD mg/g	Mean value SD mg/g
Yellow label	17.36 ± 0.41	1.86 ± 0.03	2.12 ± 0.01	4.20 ± 0.55	nd	4.54 ± 1.36

Lipton (black tea)						
Eko Oolong	18.08 ± 6.68	1.11 ± 0.37	nd	nd	4.59 ± 1.54	13.87 ± 5.00
Lipton White tea	20.51 ± 0.11	1.28 ± 0.07	nd	nd	6.62 ± 0.56	33.31 ± 1.05
Assam Molkari	24.65 ± 0.20	2.67 ± 0.03	2.66 ± 0.05	4.68 ± 0.07	8.41 ± 0.51	4.81 ± 0.24
Green Tea	20.81 ± 0.72	1.20 ± 0.01	0.64 ± 0.02	2.69 ± 0.05	7.53 ± 0.43	24.37 ± 0.47
Yellow Sunflower	16.15 ± 0.36	0.66 ± 0.01	0.85 ± 0.09	3.76 ± 0.12	5.53 ± 0.18	16.64 ± 0.39
Robios Vanlij	nd	0.29 ± 0.01	nd	nd	nd	nd

In the table 2, we see that the amount of caffeine is more concentrated in Assam tea, green tea and white tea; however it is least in the black and yellow teas. This indicates that the way of processing, planting and growing place of tea affect on the caffeine and other components in the tea. The amount of gallic acid, theobromine, catechin, epicatechin and epigallocatechin gallate is most in Assam tea. Some components were not detected (nd) in some types of tea (**table 2**). The caffeine was not detected in Robios Vanlij tea. The theobromine and catechin were not detected in Eko Oolong, Lipton White tea and Robios Vanlij teas. The epicatechin tea was not detected in Yellow label Lipton (black tea) and Robios Vanlij teas. The epigallocatechin gallate was not detected in Robios Vanlij tea.

DISCUSSION

The HPLC instrument was used for different kinds of tea to get the retention time of each type depending on the retention time of the standards. The first method which has been used [2], in which the samples were dissolved in acetonitrile: water (1:1), created some problems by showing two peaks of caffeine in the chromatogram instead of one. This problem is solved by dissolving the caffeine in water to get rid of one of the peaks and the chromatogram showed very sharp peak of caffeine.

The reason of the two peaks is that in the beginning the sample was dissolved in nonpolar solvent which was resulted as a very broad start band. By dissolving the caffeine in water, the analyte runs from polar to nonpolar medium which make the peak to be sharper and the analyte stays directly on column focusing. In the next day, the analytes were eluted in the same mobile face without preparing new one. The result was very unstable and bad peaks were obtained. The problem was solved by preparing fresh mobile phase in each day to get good peaks for the analyte.

The method of mobile phase composition which has been used in this project (water:acetonitrile:Ethyl Acetate:Methanol:Acetic acid; 89:6:3:1:1) was helpful to get rid of the double peaks and to get sharp

peaks. At the same time, by running small concentration of caffeine, the two peaks were appeared again. After checking the procedure, it is noticed that the syringe wash was acetonitrile:water (1:1). After changing it to the new mobile phase, (water:acetonitrile:Ethyl Acetate:Methanol:Acetic acid; 89:6:3:1:1), the double peaks disappeared.

CONCLUSION

The HPLC method is regarded as one of the best used methods to identify the different components of tea due to separate these components. The elution mood which is used in the method was isocratic until the minute 16 then changed to more polar to reduce the elution time and to get rid of some non-interested components in the tea solution. The using of gradient mood only to look for the components is not preferable due to the elution time needed could be more than 35 minutes. By doing the extraction at higher temperature was not too much effectible on the amount of the components of tea.

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Conflict of Interest: None

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