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Relative Analysis of Gallic-Acid, Theobromine, Theophylline, and Caffeine Content among 12 Bangladeshi Tea Genotypes Using Reversed-Phase Liquid Chromatography

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JBGU and MAR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MAA and Habiba managed the analyses of the study. Author BD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The tea industry is of great economic importance worldwide, owing to its possession of both food and medicinal values. Bangladesh is among the world-renowned tea exporting countries. However, the inadequate biochemical data for most cultivated Bangladeshi tea genotypes hinders its competitiveness on the world market. This is as a result of previous research mainly revolving

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around conventional breeding, mutagenesis, and polyploidization. This research aims to characterise the 12 Bangladeshi tea genotypes according to their biochemical content. Such information is inevitable in driving the demand and preference of these tea products on the world market.

Study Design: The study was designed based on relevant research articles and standard laboratory procedures.

Place and Duration of Study: This research was conducted at the Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh, from June 2015 to September 2016.

Methodology: Reversed-Phase Liquid Chromatography was used to determine the composition of different tea genotypes. A mixture of 999 ml de-ionised water & 1 ml TFA was used as buffer A, and 80% acetonitrile was used as buffer B in RPLC system.

Results: We found that all the 12 tea genotypes are rich in Theophylline, Theobromine, Gallic-Acid, and Caffeine content, but with varying quantities.

Conclusion: These results indicate that some of these tea genotypes can be used to produce the decaffeinated tea, a newly introduced tea product on the market that is on high demand. To ascertain the diversity of chemical composition among the various tea genotypes, biochemical characterisation of other Bangladeshi tea genotypes should be performed. Such data will enhance the market value and demand for Bangladeshi tea on the world market.

Keywords: Camellia sinensis; biochemical characterization; high-performance liquid chromatography; decaffeinated tea.

ABBREVIATIONS

HPLC : High-Performance Liquid Chromatography;
RPLC : Reversed-Phase Liquid Chromatography;
BTRI : Bangladesh Tea Research Institute;
GA : Gallic acid;
TB : Theobromine;
TP : Theophylline;
CAF : Caffeine

1. INTRODUCTION

Tea plant (*Camellia sinesis*) has been used to produce the oldest and most popular, non-alcoholic, soft beverage across the world which served as a daily drink for two-thirds of the world population. Tea also has a great medicinal value because of its polyphenol content which are great antioxidants [1]. Some of the known benefits of tea includes, improving the growth of beneficial microflora in the intestine, protecting cells and tissues from oxidative damage [2], reducing tumors and mutations [3], promoting anti-oxidant and antimicrobial activity [4], preventing dental caries [5], preventing cardiovascular disease [6], lowering blood cholesterol [7], inhibiting the increase of blood sugar and blood pressure [8], killing bacteria and influenza virus [9], and increasing thermogenesis and bone density [10]. In addition, tea works as therapeutics on patients suffering from several debilitating diseases, as well as provides health-giving food supplements for the general population [11].

Commercial tea cultivars are commonly found as three different taxa, namely, *C. sinensis*, *C. assamica*, and *C. assamica ssp. lasiocalyx* [12]. Though tea is a highly heterogeneous plant [13] and all three above mentioned taxa are freely inter-breed, they result in a line extending from extreme China types to those of Assam origin [14]. Typically, tea leaves contain alkaloids (caffeine, theophylline, theobromine etc.) (Fig. 1), polyphenols, amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride minerals, and trace elements. Furthermore, tea contains 4000 bioactive compounds, of which one-third is polyphenols [15].

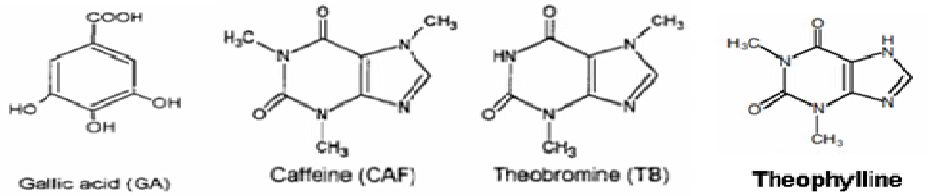


Fig. 1. Structure of Gallic Acid and some Alkaloids (Caffeine, Theobromine, and Theophylline) available in tea

At the beginning of the 21st century, it was reported that tea is being consumed more than 3 billion cups/day [16] and the consumption rate is increasing day by day. So, obviously that the tea industry is growing and has already become a huge global market of significant economic importance. At present, the continent of Asia is leading the race of tea production with 83% of the total, and among its affiliates, China and India are prominent with 28.9% & 26.2% of total production, respectively [17].

Industrial production of tea is not new in Bangladesh which produces 1.6% of total tea production. Although the amount is low, still Bangladesh manages to export a considerable amount of tea from what it produces (i.e. 0.6 % of total tea exports). In this competitive global tea market, only increasing the production of tea is not enough to attract buyers. In present days, tea importing countries want to buy authenticated tea products with a well-known chemical composition of that tea which they are buying. The history of tea research in Bangladesh is not new. Tea research in Bangladesh has been in existence even before the birth of Bangladesh. A Pakistani Scientist named Ahmed earlier distinguished the tea population of Bangladesh (by then known as East Pakistan) into 6 eco and geo categories but till now in Bangladesh tea research is evolving around only breeding, mutation, and polyploidization approaches. As a result, there is no data available regarding the chemical composition of the different tea genotypes from Bangladesh. So far, the only success in tea research of Bangladesh is the development of 18 high yielding tea clones [18]. The purpose of our research was to find out the chemical composition of different tea genotypes most commonly found/cultivated in Bangladesh and to identify the appropriate genotype for the production of decaffeinated tea.

The word 'Decaffeinated' refers to a product that was originally containing caffeine following the removal of most of its caffeine. Thus, decaffeination certainty does not mean the absolute removal of caffeine. In tea, decaffeination can be done by using chloroform or methylene chloride but this method is not widely accepted by consumers because of the toxicity it generates [19]. Other available and healthy ways of decaffeination are using supercritical carbon dioxide [20], ethyl acetate decaffeination, using sawdust lignocellulose columns [19] and decaffeination of fresh green tea leaf by hot water treatment [21]. By law, tea labeled as 'decaffeinated' needs to have less than 2.5 percent of its original caffeine level.

To produce the decaffeinated tea, it is essential to choose the right tea genotype because tea consumers are preferring decaffeinated tea, do not want to consume much caffeine. As decaffeinated tea contains nearly 2.5% of its original caffeine level, choosing the genotype which already has less caffeine is very important. In our study, two released and popular tea genotypes (BT-2 and Bt5) from BTRI were used to compare with 10 unreleased tea genotypes. All these tea genotypes were high yielding and cup quality of these genotypes was above average to excellent [21,22].

High-Performance Liquid Chromatography (HPLC) is one of the most widely used techniques for identification, quantification, and purification of mixtures of organic compounds. If in HPLC, the stationary phase is less polar than the mobile phase then it is known as Reversed-Phase Liquid Chromatography (RPLC). In RPLC, C3, C8, or a C18 column are used as stationary phase, while the mobile phase is a polar solvent like methanol, acetonitrile, etc. or mixtures with water. It is the most common technique in liquid chromatography and thus used in the current project for biochemical profiling.

We hypothesised that all the genotypes used in this research have GA, TP, TB, and CAF but varying in content. Another hypothesis of our research was to find at least one unreleased tea genotype having lower caffeine content in comparison to released tea genotype BT-2 and Bt5. This research would certainly provide a foundation for the biochemical analysis of Bangladeshi tea genotypes which may inspire others to work on biochemical analysis using all the available tea genotypes of Bangladesh.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples and Estimation of the Cup Quality

Green tea leaves from 12 different (Ph/9/25, MZ/39, E/4, D/13, Br2/97, Ph/9/4, SDL1, Bt5, B2xT1, BT2, Ph/9/40, BS67) genotypes (Fig. 2) were collected from Bangladesh Tea Research Institute (BTRI). Top two leaves and buds of each genotype were used to produce the green tea powder. After plucking, tea leaves were

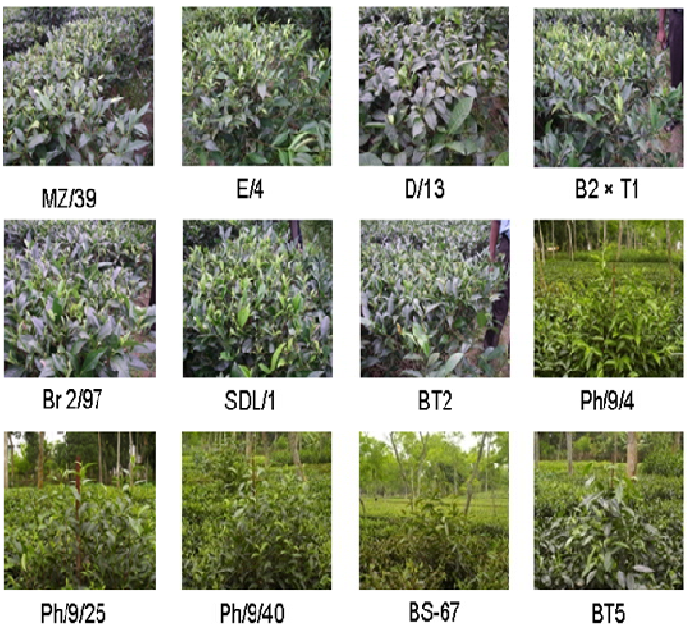


Fig. 2. 12 Bangladeshi tea genotypes used for biochemical characterisation

washed thoroughly with clean water and then dried by steaming for 150 seconds to prevent fermentation. Avoiding fermentation was necessary as it stopped enzyme activities that might cause oxidation. Steaming was conducted on a bamboo tray over water. Then those tea leaves were cooled and dried at 34-36°C overnight to blow water from the leaves. The cooled and dried leaves were then hand-rolled to create a uniformly rounded leaf. During rolling, the temperature was maintained at 34-36°C to ensure the quality of tea. The blending of rolled tea leaves was performed using "NutriBullet Pro 900" blender machine to produce green tea powder.

After finishing processing, tea samples (20 g from each accession) were sent for tasters' evaluation. The rest of the tea powders were kept in sealed packets in a refrigerator at 4°C for further use. The sensory evaluation made by three professional tea tasters with scores based on infused leaf color, the color of liquor, briskness, strength and creaming down (thickening of the liquor) was used to determine the parameters related to the quality of tea liquor. Each parameter was scored based on a scale of 10. Thus, the overall tea tasting consisted of a score of 50. The tea that got a total score ranging from 34 or above, 32 to below 34, 30 to below 32, and below 30, were remarked as excellent (E) tea, above average (AA) tea, average (A) tea and below average (BA) tea, respectively.

2.2 Analysis of Gallic Acid (GA) and Significant tea Alkaloids Using Reverse Phase Liquid Chromatography (RPLC)

Among different chromatographic techniques, the Reverse Phase High-Pressure Liquid Chromatography (RPLC) was used in our investigation. Gallic acid (GA) and major tea alkaloids were analyzed using RPLC (Waters HPLC system, USA consisting of two 515 HPLC pump, pump control unit and 2489 UV/detector). The standards of Gallic acid, theobromine, theophylline, caffeine, (-) were obtained from Sigma-Aldrich, USA. For RPLC, C18 column (Particle size: 5 μm, Pore size: 300 Á, Dimension: 250 mm × 10 mm) was used as a non-polar stationary phase. In the mobile phase, two buffer solutions (i.e. buffer A and buffer B) were used whose details are given in the following section.

2.2.1 Preparation of buffer A and buffer B for RPLC

One liter of Buffer A was prepared using 999 mL of de-ionised water. 1 mL of TFA was added to it and mixed by swirling. It was then filtered through a 0.45 μ PTFE membrane using a Sartorius vacuum buffer filtration system. The buffer was stored at room temperature while not in use. 80% acetonitrile was used as buffer B for

the current experiment. To make 1 L of buffer B, 800 mL of acetonitrile was mixed with 199 mL of water and 1 mL of TFA was added to it. Buffer B was also filtered and stored in the same way as buffer A.

2.2.2 Equilibration of the RPLC system and column

The whole system including the pump, tubing, and sample loop was washed with 100% buffer A. The C18 column was connected to the RPLC and first, it was washed with 100% buffer B until the baseline monitored at 280nm was stable as well as to remove any residual protein attached to it. Two Column volumes (CV) (4 mL × 2 = 8 mL) of buffer B was needed to pass through the column for complete washing. Then the column was equilibrated with three CV (4 mL × 3 = 12 mL) of 100% buffer A. The flow rate was set as 1ml/min during equilibration, which was done until the baseline became linear.

2.2.3 Sample preparation and injection

For sample preparation, we used tap water, because usually distilled and other processed water is not used to make a cup of tea. The procedure of sample preparation was inspired by the way of making a cup of tea in Bangladesh. The procedure is discussed in the following paragraph.

At first, 70 ml of water was boiled and precipitation was removed. Then, from this 70 mL water, nearly 50 ml water was taken to be boiled again. Afterward, 0.6 g of dried powdered tea sample was added and mixed vigorously in boiled water. Dried powdered tea sample containing boiled water was kept in boiling temperature for a minute. After that, water extract of tea sample was kept in room temperature for a couple of minutes to let it be cooled. Then, that water extract was filtered in two steps firstly using filter paper, secondly using 0.45μ PTFE syringe filtration membrane. After filtration, 1 mL of each tea sample was transferred into labelled 1.5 mL Eppendorf tube.

The above procedure was applied to the above mentioned all fresh leaf samples of 12 tea genotypes. 20 µL of each of these extracts were injected into the RPLC system. Standards were also run in a volume of 20 µL using the same RPLC protocol. A sample loop of 20 μL was used in the experiments and the samples were subjected to C18 reverse phase RPLC column. Elution was monitored at UV absorption at 280 nm.

2.2.4 Gradient elution

For the elution of proteins, the concentration of buffer B was increased gradually. As the binding affinity of the proteins depends on the hydrophobicity, it is expected that the proteins which are loosely bound to the column (less hydrophobic and more polar) will elute in less amount of buffer B from the column whereas the more hydrophobic proteins will need more concentration of buffer B to release from the column. Initially, 100% buffer was passed through the system and then gradually percentage of buffer A was decreased and buffer B was increased. The gradual increase of buffer B with the decrease of buffer A was maintained in a mixing chamber of the RPLC system and pumped in the system through the column. For RPLC purification of 12 tea cultivars we developed a protocol as follows:

100%A	:	1 CV
0 - 30% B	:	1 CV
30 - 80%B	:	5 CV
80 - 100%B	:	1 CV
100%B - 100%A	:	1CV
100% A	:	1CV
20% Ethanol	:	1 CV

2.3 Data Analysis

One-way analyses of variance (ANOVA) with Tukey's test at 5% level of significance was used to determine the difference between standard and content values (unreleased tea genotypes) using SPSS Statistical Package (SPSS 13.0, SPSS Inc., IL, U.S.A.). We used the average %Area value of released tea genotypes (BT-2 and Bt5) as standard.

3. RESULTS

3.1 Determination of Gallic Acid, Theobromine, Theophylline and Caffeine through RPLC

4 standards were compared with the chromatograms produced by the 12 tea genotypes leaf extracts. To identify Gallic acid, theobromine, theophylline, and caffeine, standards were run first. All the standards gave a single curve in the chromatogram. Gallic acid (standard) peak appeared at approximately 14th minutes (Fig. 3a). Theobromine, theophylline,

and caffeine (standard) peak appeared at approximately 18th, 19th and 24th minute respectively (Fig. 3b, Fig. 3c, Fig. 3d). Each chromatogram produced by different tea genotypes showed several numbers of peaks and all of the 4 standards were present in all 12 tea genotypes but their quantities were different (Fig. 3).

3.2 Study of Different Chromatograms Obtained from Different tea Genotypes

All of our used tea genotypes scored above average to excellent in cup quality test. That's why, we used all those genotypes to analyze Gallic acid, Theobromine, Theophylline, and Caffeine content. In chromatogram, different tea genotypes generated a different number of total peaks. The chromatogram generated from the tea genotype Ph/9/25, MZ/39, E/4, D/13, Br2/97, Ph/9/4, SDL1, Bt5, B2xT1, BT2, Ph/9/40, BS67 showed 43, 49, 53, 48, 54, 45, 50, 51, 49, 44, 52, 56 peaks respectively (Fig. 4). As each peak indicates the presence of a particular biochemical element, the variation of total peak number indicated that each genotype is having a different number of the biochemical element which is an evidence of their biochemical variation from each other.

In our study, we mainly focused on four very important component of tea which are vital to determine the tea quality. Those four biochemical component were: Gallic acid, theobromine, theophylline, and caffeine. In each chromatogram, we observed peaks in our expected retention time's regions which indicated the presence of these chemicals in each Bangladeshi tea genotype (Fig. 4).

Though the RPLC system and the chromatographic condition was same to each tea genotype, the retention time of Gallic acid, theobromine, theophylline, and caffeine slightly varied from genotype to genotype (Table 1). But this little variation is acceptable, because every time Gallic acid, theobromine, theophylline, and caffeine appeared very nearly to the retention time, which we observed while running the standards only (Fig. 3). In every single tea genotype, the retention time of GA was lowest

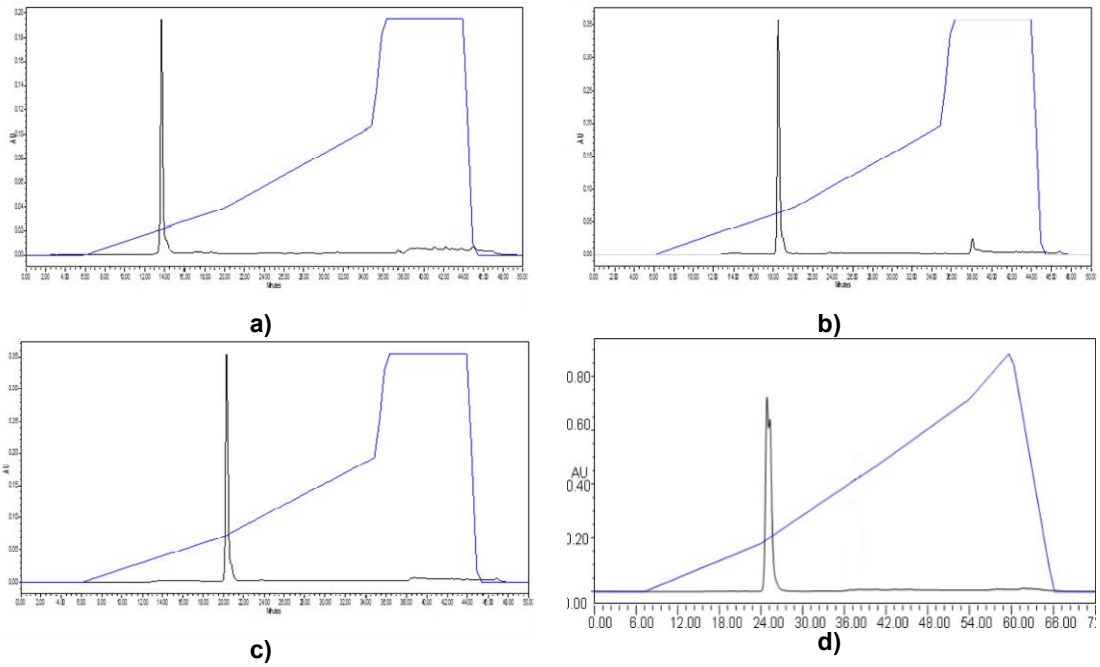


Fig. 3. RPLC chromatogram of aquatic leaf extract of different standards at 280 nm. The X-axis represents retention time and the Y-axis represents absorbance. The sample was run on 100 C18 5µ 25 × 0.46 column using a mixture of 1 mL TFA and 999 mL water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 mL/min. Major peaks were marked by the standard run using the same chromatographic condition.

Here, Fig. a, b, c, d is respectively representing the chromatogram of Gallic acid, Theobromine, Theophylline, and Caffeine standard.

which was near to 14th minutes. On the other hand, the retention time of CAF was highest in each genotype (Fig. 4, Table 1).

The most important and noticeable change was observed in the value of %Area which is the main parameter to understand the concentration of each chemical in different tea genotypes (Table 1). Though the value of %Area varied from chemical to both chemical and genotype to genotype. In every genotype, the %Area value of theophylline was lowest and caffeine was highest (Table 1). It indicated that all these tea genotypes are having a good amount of caffeine but their theophylline concentration is low. To better understand the actual biochemical variation among all these genotypes, we performed a comparative analysis.

Among the 12 genotypes, Bt-5 showed the highest percentage of Gallic acid followed by Ph/9/25, BT2, BS67. On the other hand, B2xT1 showed the lowest amount of Gallic acid followed by SDL/1, D/13, and Ph/9/40 (Table 1). In case of TB, Ph/9/40 showed the highest performance with the value of 3.95% of theobromine followed by E/4 and BT2, whereas, genotype BS-67 showed the lowest amount of theobromine content followed by Ph/9/4, SDL/1 (Table 1). The tea genotype B2xT1 showed the highest amount of theophylline content followed by MZ/39 and D/13. The genotype Ph/9/4 showed the lowest theophylline content followed by Ph/9/40, Ph/9/25, and BT2 (Table 1). Out of 12 tea genotypes, BT2 showed the highest caffeine content followed by Bt5, Ph/9/25, whereas the genotype Br2/97 showed the lowest amount of caffeine content followed by SDL/1 and B2×T1 (Table 1).

3.3 Comparative Study of RPLC Analysis

Four biochemical components viz., Gallic acid (GA), theobromine (TB), theophylline (TP), caffeine (CAF) were analysed in 12 different tea genotypes through RPLC to find out the quantitative differences among those genotypes. Our analysis showed that the GA content of tea genotypes D/13, SDL1, B2xT1, Ph/9/40 was varied significantly from the standard value. While comparing the standard TB value with other tea genotypes, it showed that Ph/9/4, SDL1, and BS67 were varied significantly in TB content. Tea genotype B2xT1 contained a high level of TP which was significantly different from the standard value of TP content. Low caffeine-containing tea genotype, Br2/97, and B2xT1 showed significance level of variance to the standard value of caffeine content in one-way ANOVA and t-test.

Table 1. Retention time, area covered and % area of different peaks obtained in the RPLC of aquatic leaf extract of different Bangladeshi tea genotypes.

Name of Tea Genotype	Name of the chemical	Retention time	Area covered	%Area
Ph/9/25	Gallic acid	13.989	2396731	7.73
	Theobromine	18.117	784528	2.53
	Theophylline	19.489	119483	0.39
	Caffeine	22.78	14248954	45.97
MZ/39	Gallic Acid	13.908	5203270	5.11
	Theobromine	18.084	1280326	1.26
	Theophylline	19.433	854545	0.84
	Caffeine	22.741	35144875	34.51
E/4	Gallic Acid	13.872	2599984	5.53
	Theobromine	18.049	1708206	3.63
	Theophylline	19.382	308755	0.66
	Caffeine	22.694	20737898	44.12
D/13	Gallic Acid	13.866	2058194	4.45
	Theobromine	18.047	998109	2.16
	Theophylline	19.404	345808	0.75
	Caffeine	22.734	15996306	34.58
Br2/97	Gallic Acid	13.744	4924394	6.01
	Theobromine	18.205	1225908	1.5
	Theophylline	19.609	461983	0.56
	Caffeine	23.006	24685672	30.11
Ph/9/4	Gallic Acid	13.755	3508330	5.9
	Theobromine	18.267	627656	1.06
	Theophylline	19.723	158420	0.27

Name of Tea Genotype	Name of the chemical	Retention time	Area covered	%Area
	Caffeine	23.173	22162203	37.27
SDL1	Gallic Acid	13.77	1963338	4.33
	Theobromine	18.26	508861	1.12
	Theophylline	19.715	157439	0.35
	Caffeine	23.171	15507173	34.16
Bt5	Gallic Acid	13.763	1905616	8.47
	Theobromine	18.24	442289	1.97
	Theophylline	19.592	154915	0.69
	Caffeine	23.158	10787103	47.96
B2xT1	Gallic Acid	13.749	3194665	4.07
	Theobromine	18.229	2120702	2.7
	Theophylline	19.64	894490	1.14
	Caffeine	23.134	25405765	32.36
BT2	Gallic Acid	13.752	2124380	7.64
	Theobromine	18.291	992796	3.57
	Theophylline	19.45	111603	0.4
	Caffeine	23.21	13863902	49.86
Ph/9/40	Gallic Acid	13.88	3503434	4.67
	Theobromine	18.572	2965271	3.95
	Theophylline	19.7	232229	0.31
	Caffeine	23.703	25675418	34.22
BS67	Gallic Acid	14.303	2897743	7.21
	Theobromine	17.986	420440	1.05
	Theophylline	19.647	230358	0.57
	Caffeine	22.738	14783047	36.79

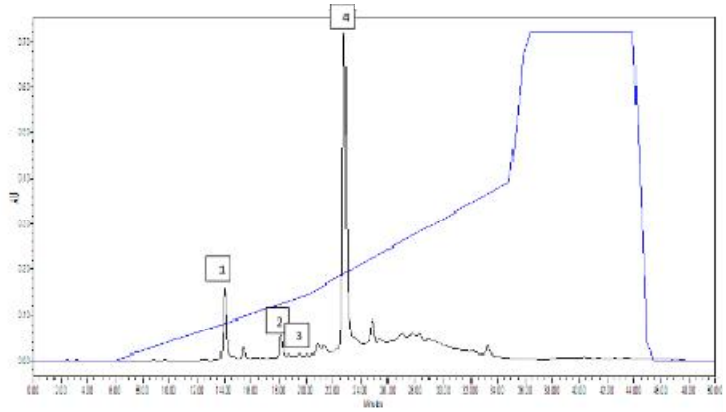
4. DISCUSSION

Nutritional therapy and phytotherapy are newly emerged concepts of health aid. Because of presumed safety and potential nutritional-therapeutic effects of a plant-derived nutraceutical or functional foods, these types of foods are receiving a considerable amount of attention among health-conscious consumers. As major tea contents like alkaloid (TP, TB, CAF etc.), tannins, steroids, flavonoids have both medicinal and food value [22], nowadays tea is not being considered only as a food product, but also as a medicinal product. So, consumers of this time, prefer to have both taste and health at a time by drinking tea. Because of not having the proper chemical composition of different Bangladeshi tea genotypes, previously it was not that much-preferred product to the global health-conscious consumers of tea.

Geographical location and the prevalent environmental specification (both agronomic and pedoclimatic) hugely affect the phytochemical characteristics of tea cultivars [23]. As there was no data available on the biochemical characterisation of Bangladeshi tea genotypes, tea industry of Bangladesh used to face a challenge to establish their product as an authenticated product in the world tea market. This study is the first step in exploring the biochemical content of different tea genotypes from Bangladesh.

Our study showed that all 12 Bangladeshi tea genotypes are having TP, TB, and GA. This study also showed that Bangladeshi tea genotypes are rich in CAF content among 12 tea genotypes, BT2, Bt5, and Ph/9/25 are having a high amount of caffeine which may attract caffeine loving consumers.

But at the same time, few consumers are being reported to have an allergic reaction to caffeine [24]. Notable problems they suffer by consuming caffeine includes diarrhea, dizziness, fast heartbeat hyperglycemia, blurred vision, drowsiness, dry mouth, flushed dry skin, fruit-like breath odor, increased urination, ketones in urine, loss of appetite, nausea, stomachache, tiredness, troubled breathing, unusual thirst, or vomiting (in newborn babies), hypoglycemia, including anxious feeling, cold sweats, confusion, cool pale skin, drowsiness, excessive hunger, fast heartbeat, nausea, nervousness, restless sleep, shakiness, or unusual tiredness or weakness (in newborn babies), irritability, nervousness, severe jitters (in newborn babies), tremors, trouble in sleeping and vomiting.



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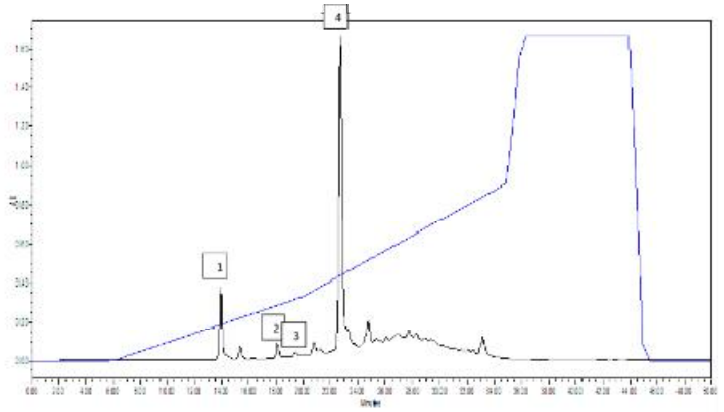

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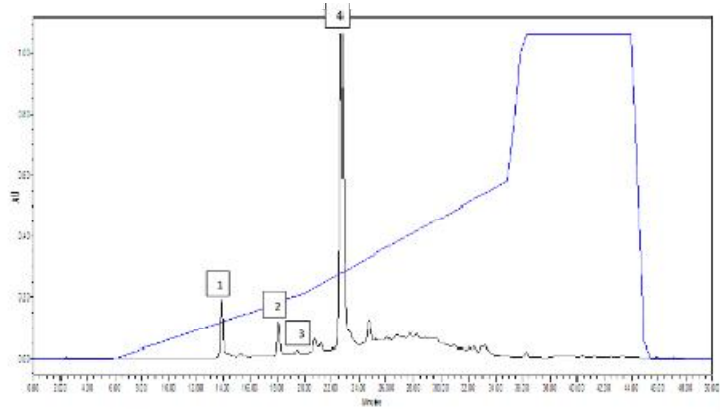

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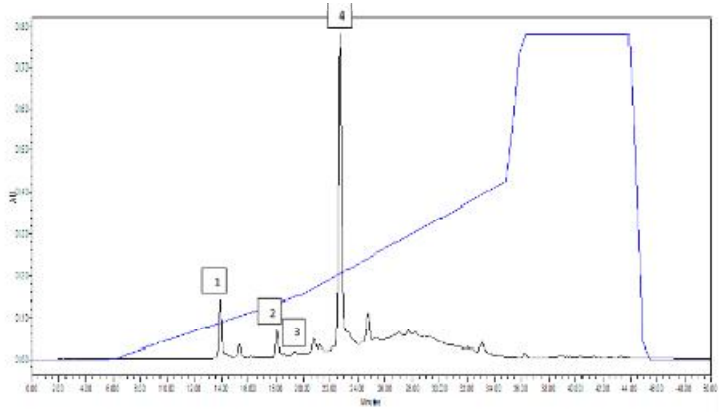

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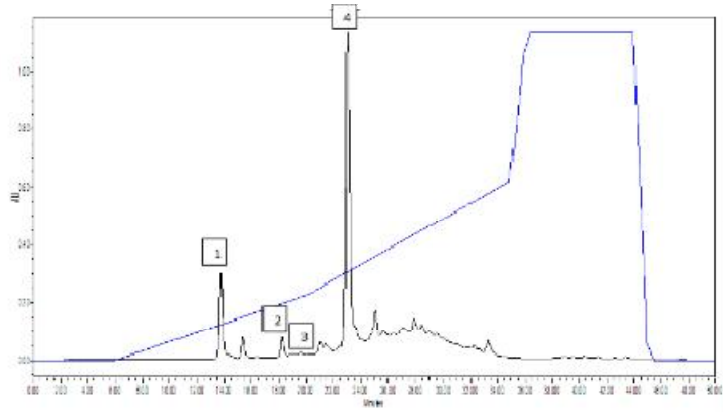

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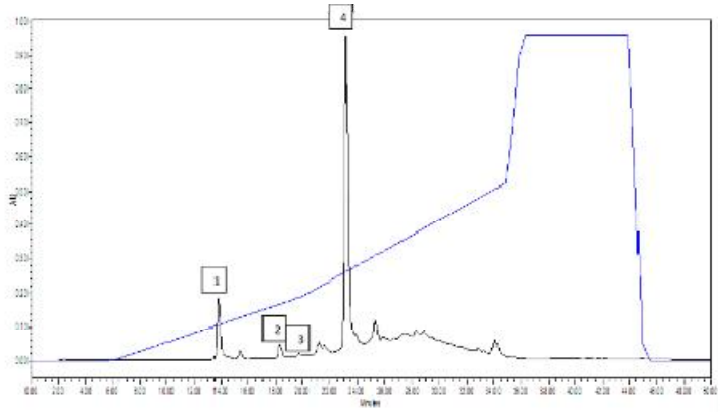

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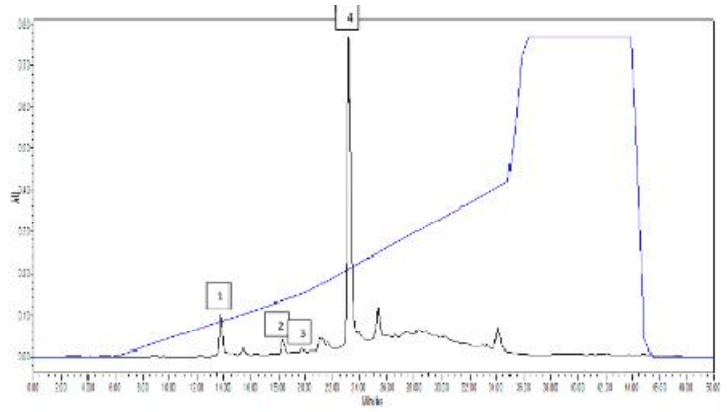
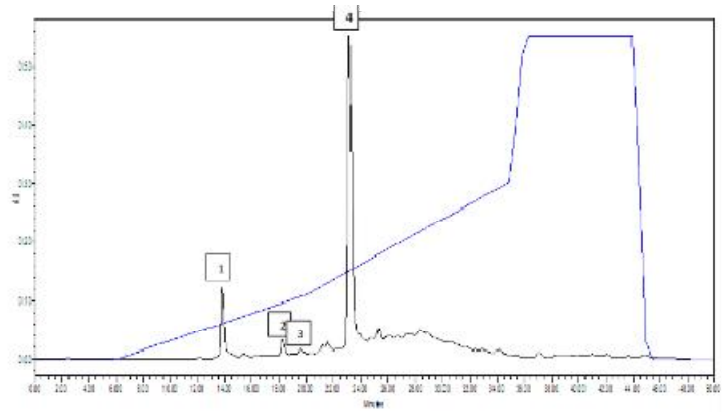


g)



h)

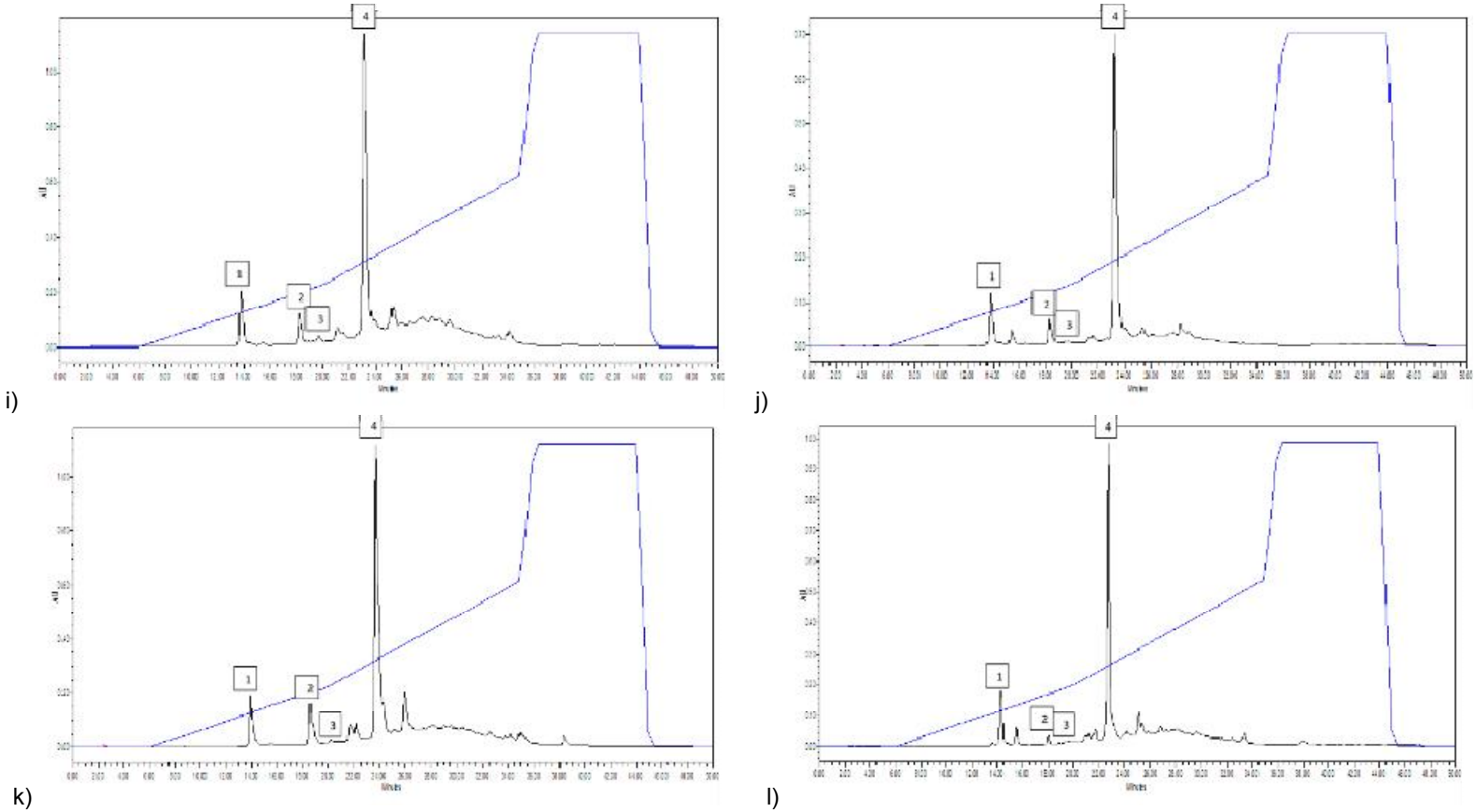


Fig. 4. RPLC chromatogram of aquatic leaf extract of different tea genotypes at 280 nm. The x-axis represents retention time and the Y-axis represents absorbance. The sample was run on 100 C18 5µ 25 × 0.46 column using a mixture of 1 mL TFA and 999 mL water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 mL/min. Major peaks were marked by the standard run using the same chromatographic condition. Labelling 1,2,3,4 represent Gallic acid, theobromine, theophylline, caffeine respectively.

Here, figure a, b, c, d, e, f, g, h, I, j, k, l is representing the chromatogram of Tea Genotype Ph/9/25, MZ/39, E/4, D/13, Br2/97, Ph/9/4, SDL1, Bt5, B2xT1, BT2, Ph/9/40, and BS67 respectively.

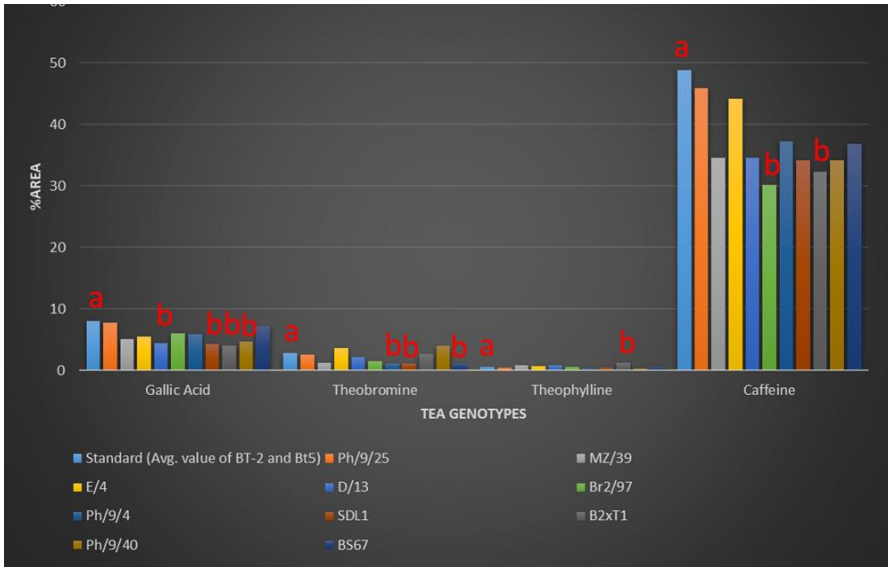


Fig. 5. Comparative study of RPLC analysis (comparison of standard and content values). The values are means of percent area of biochemical contents, [a,b]different letters in the same column are significantly different at p = 0.05 level

So, the demand for decaffeinated tea is increasing day by day. There are few decaffeination processes available to produce decaffeinated tea but most of these decaffeination processes are having few issues related to health, production expense or quality concern. Such as few decaffeination processes are toxic [19], some are expensive [20] and some are lessening the catechin content [21] which have the capability to suppress excess free radicals of the human body [25], thus directly related with the tea quality [26]. That is why it is very important to choose the right tea genotype to produce decaffeinated tea. Our data is giving that information about choosing the appropriate tea genotype for this purpose. Some consumers also prefer to have naturally less caffeine-containing tea (i.e., not decaffeinated). Maybe, Bangladeshi tea genotype Br2/97 and B2xT1 are the right choices of products for them.

Activities of total alkaloid, phenolic, flavonoids, and antioxidants are the parameters depicting the quality of tea according to their biological properties [27]. China and India are the top two tea producing countries. In the world tea market, the demand for Chinese and Indian tea is very high. Previous reports showed that 22 Chinese tea cultivars are rich in TB, GA and CAF [28], though it was not disclosed that how much TP those 22 tea cultivars were containing. On the other hand, previous reports showed that Indian tea cultivars are rich in alkaloid content [23], but the individual amount of different alkaloids in those cultivars were not properly described. Our studies showed that Bangladeshi tea genotypes are having all the necessary alkaloids as Chinese and Indian tea.

We conducted this research to find out whether these 12 Bangladeshi genotypes are having TP, TB, GA, and CAF or not. Our study showed that all these Bangladeshi tea genotypes contain TP, TB, GA, and CAF and their amount varies from genotype to genotype. Two main objectives of our research were to find out the biochemical content of different Bangladeshi tea genotypes and to select the appropriate Bangladeshi tea genotype to produce decaffeinated tea. Our research fulfilled both the objectives. Given the promise of the outcome of this research, further biochemical analysis using other Bangladeshi tea genotypes is recommended.

5. CONCLUSION

Till present, breeding approaches based on morphological appearances were the most used method of tea improvement in Bangladesh. Our study proved that biochemical analysis can play a vital role in tea improvement program. Prior to breeding, biochemical approaches can be useful to find out the distantly related tea genotypes. At the same time, biochemical characterization can be performed for quality evaluation of both newly developed and existing tea genotypes and

varieties. Moreover, the improvement program using biochemical content is much faster than the breeding program. The main hindrance on the path of tea improvement program in Bangladesh was lack of baseline data on biochemical contents of different tea genotypes which we tried to resolve by providing data on 12 Bangladeshi tea genotypes. This biochemical characterisation is the first step of establishing a database on biochemical composition of different Bangladeshi tea genotypes which can help to evaluate the tea quality of Bangladesh by determination and quantification of different biochemical compounds. Moreover, as the results of this study demonstrated that different Bangladeshi tea genotypes vary from each other in caffeine content, these results can be used to produce the decaffeinated tea which is a newly emerged product in demand.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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