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Original Article

# GC–MS based metabolite profiling and angiotensin I-converting enzyme inhibitory property of black tea extracts

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ABSTRACT

Angiotensin I-converting enzyme inhibitors are used as therapeutic agents for the treatment of hypertension. Regular consumption of black tea (*Camellia sinensis* (L.) Kuntze, Theaceae) has been reported to lower blood pressure. The aims of the present work were to compare chemical composition and angiotensin I-converting enzyme inhibitory properties of infusion and decoction of four samples of black tea. GC/MS based metabolomics approach helped in identification of fifty-one metabolites including ten organic acids, one inorganic acid, sixteen amino acids, two sugars, five sugar alcohols, fifteen phenols and flavonoids, two fatty acids from infusions and decoctions of four black tea samples. Partial least squares discriminant analysis and orthogonal partial least squares discriminant analysis models showed good classification among the two groups, diffusion and infusion, based on metabolites. Both infusion and decoction inhibited the enzyme. However, the activity differed with samples. Multivariate analysis also segregated extracts on the basis of activity. Thearubigin, theaflavin, catechin inhibited the enzyme. Epicatechin, epigallocatechin gallate, gallic acid, caffeine showed lower activity.



## Introduction

Tea, produced from the top two fresh green leaves and a bud of *Camellia sinensis* (L.) Kuntze, Theaceae, is one of the most frequently consumed beverages in the world. Tea is generally classified as black tea, green tea, oolong tea and white tea depending on the degree of maceration of leaf cells and fermentation during the manufacturing process (Hilal and Engelhardt, 2007). Black tea (BT) accounts for 72% of the world's total tea production (Ng et al., 2008). Medicinal and therapeutic potential of BT (Sharangi, 2009) is well known.

Angiotensin I-Converting Enzyme (ACE) is a key component in the renin angiotensin aldosterone system (RAAS) (Balasuriya and Rupasinghe, 2011) which converts inactive angiotensin I to angiotensin II, a powerful vasoconstrictor and promoter of sodium retention, and inactivates vasodilator bradykinin which is conducive to lower blood pressure (Johnston and Franz, 1992). One of the reasons of the pathogenesis of hypertension is increased activity of RAAS (Balasuriya and Rupasinghe, 2011). In many cardiovascular diseases, angiotensin II is present in abnormally high quantities. Blocking production of angiotensin II with ACE inhibitors prevents constriction of blood vessels, lowers blood pressure (Sweitzer, 2003). ACE inhibitors were developed as therapeutic agents for the treatment of hypertension (Brown and Vaughan, 1998). Several ACE inhibitors such as captopril, enalapril, lisinopril and temocapril are in clinical use for the treatment of hypertension. All of these drugs produced side effects, thus justifying the search for natural ACE inhibitors for safe and economical use (Coates, 2003). Therefore, ACE inhibitors from natural sources, particularly dietary sources, are potentially beneficial. Different plant based foods (Miccue et al., 2005; Oboh et al., 2012; Das and De, 2013; Acharya et al., 2016), other plants (Braga et al., 2007), phenolic rich extracts of soybean (Ademiluyi and Oboh, 2013) and several phytochemicals such as isoquercetin, apigenin, luteolin, glycosides of quercetin, cyanidin, kaempferol, luleolin (Loizzo et al., 2007; Junior et al., 2011; Balasuriya and Rupasinghe, 2011) were reported to inhibit ACE.

Regular consumption of black tea has been reported to lower blood pressure (Hodgson et al., 2012). *In vitro* ACE inhibitory activity was affected by the tea processing method with $IC_{50}$ values for ACE inhibition: green < oolong < white < black < dark teas. The report suggested that the tea polyphenolics exert a mixed mode of *in vitro* inhibition of ACE (Dong et al., 2011).

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In India black tea is consumed either as infusion or as decoction (Ray et al., 2014). The aims of the present work were to compare chemical composition and ACE inhibitory properties of infusions and decoctions of different samples of black tea.

## Materials and methods

### *Plant materials*

Four black tea – *Camellia sinensis* (L.) Kuntze, Theaceae – (BT) samples (Doors tea, Siliguri tea, Guwahati tea and Nilgiri tea) (CTC, first flush) were collected in 2006 from Duncans Tea Ltd., Kolkata, India who are having their own tea gardens and they are also in packaged tea business for retail consumers where tea is collected from different sources. The samples were identified by B. De and are available in the laboratory.

### *Reagents*

Theaflavin [80% theaflavins (theaflavin and theaflavingallates)], angiotensin converting enzyme from rabbit lung, hippuryl-L-histydyl-L-leucine (HHL), catechin, epicatechin, epigallocatechingallate, methoxyamine hydrochloride, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), methyl octanoate (C8), methyl decanoate (C10), methyl laurate (C12), myristic acid methyl ester (C14), methyl palmitate (C16), stearic acid methyl ester (C18), methyl arachidate (C20), methyl behenate (C22), methyl tetracosanoate (C24), methyl hexacosanoate (C26) were purchased from Sigma–Aldrich (St. Louis, MO); gallic acid, caffeine, from Sisco Research Laboratories Pvt. Ltd., India. All the solvents used for GC/MS were of HPLC grade. Other reagents were of analytical grade.

### *Preparation of extract*

Infusion of tea was prepared by soaking tea leaves (20 g) in boiled distilled water (170 ml) for 5 min. The aqueous filtrate was evaporated to dryness under reduced pressure. Decoction of tea was prepared by boiling tea leaves (20 g) in distilled water (170 ml) for 5 min. The aqueous extract was filtered and evaporated to dryness. The dried infusion and decoction of BT was stored at −20 °C.

### *Preparation of thearubigin*

Thearubigin (TR) was isolated from BT following the previously reported method (Misra et al., 2003). BT (6 g) was boiled in 50 ml sodium acetate (10 mM, pH 5.0) for 10 min, cooled and filtered. The filtrate was extracted successively with equal volumes of chloroform, methyl isobutyl ketone and ethyl acetate. The organic layers were discarded and the aqueous layer was extracted with butanol followed by lyophilization. The residual dark orange powder constituted the TR.

### *GC–MS analysis*

Crude BT extract was derivatized and analyzed by GC–MS following the previously reported method (Das et al., 2016; Karak et al., 2017). Briefly, BT extract and adonitol (internal standard) dissolved in MeOH:$H_2O$ (1:1) was distributed into Eppendorf tubes (4 × 50 μl) and evaporated to dryness. The residue was re-dissolved in 10 μl of methoxyamine hydrochloride (20 mg/ml in pyridine) and subsequently shaken for 90 min at 30 °C and then 90 μl of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was added and shaken at 37 °C for 30 min for trimethylsilylation of acidic protons to increase volatility of metabolites. 2 μl FAME (fatty acid methyl esters) markers (a mixture of internal retention index (RI) markers) dissolved in chloroform was added. GC–MS analysis (Agilent 5975C Gas Chromatography System) was carried out in HP-5MS capillary column (Agilent J & W; GC Columns, USA) (length 30 m plus Duraguard 10 m, diameter 0.25 mm narrow bore, film 0.25 μm). The analysis was performed under the following oven temperature programme: oven ramp 60 °C (1 min hold) to 325 °C at 10 °C/min, 10 min hold before cool – down, 37.5 min run time. The injection temperature was set at 250 °C; the MS transfer line at 290 °C and the ion source at 230 °C. Helium was used as the carrier gas at a constant flow rate of 0.723 ml/min (carrier linear velocity 31.141 cm/s). Sample (1 μl) was injected via the split mode (split ratio 1:5) onto the GC column. Automated mass spectral deconvolution and identification system (AMDIS) was used to deconvolute GC–MS results and to identify chromatographic peaks. Identification of the metabolites was carried out by comparing the fragmentation patterns of the mass spectra and retention times (Rt) with those present in Agilent Fiehn Metabolomics library using Agilent retention time locking (RTL) method.

### *Assay of angiotensin converting enzyme*

Angiotensin converting enzyme (ACE) inhibitory activity was measured following the spectrophotometric assay (Cushman and Cheung, 1971) with modifications. Aqueous tea extract (15 μl)/its component (37 μM) was incubated with 250 μl of 200 mM borate buffer (pH. 8.3) containing 2 M NaCl and 7 mM hippuryl-histidyl-leucine (HHL). ACE from rabbit lung, (100 mU) (15 μl) and distilled water (20 μl) were added and the reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped with 1 N HCl (250 μl). The hippuric acid formed was extracted with ethyl acetate (1500 μl). After removal of ethyl acetate (1000 μl) by evaporation, hippuric acid was dissolved in 500 μl of distilled water and measured spectrophotometrically at 228 nm. The activity of each sample was tested in triplicate. The percentage inhibition of ACE activity by tea extract and its components were calculated.

### *Statistical analysis*

Multivariate analysis was performed on the normalized data set using SIMCA software. Groups were formed based by activity, infusion and decoction. Unsupervised model such as principal component analysis (PCA) and supervised models such as orthogonal partial least square discriminant analysis (OPLS-DA) were generated. R2 (goodness-of-fit) and Q2 (goodness-of-prediction) values were extracted for validation of these models.

## Results and discussion

### *Analysis of chemical composition*

Different groups of chemical constituents have previously been reported from BT. They include alkaloids (Hilal and Engelhardt, 2007), hydrolysable tannins, simple phenols and derivatives (Sakamoto et al., 2012; Ramalho et al., 2013), catechins, theaflavins (Hilal and Engelhardt, 2007; Li et al., 2013), thearubigins (Drynan et al., 2010), other flavonols and glycosides (Degenhardt et al., 2000), amino acids (Alcazar et al., 2007; Sakamoto et al., 2012) and volatile and non-polar constituents (Sereshti et al., 2013; Wang et al., 2011). Thearubigins are complex in nature. Estimated 60% of the solids in BT infusion are thearubigins, with more than 5000 individual chemical entities (Kuhnert, 2010). In our previous work, we reported presence of gallic acid, catechin, epicatechin, epicatechingallate, epigallocatechin gallate, theaflavin, theaflavin-3-monogallate, theaflavin-3′-monogallate, and theaflavin-3,3′-digallate in both infusion and decoction of the

**Table 1**
Metabolites detected in BT extracts.

| Metabolites | Doors | | Guahati | | Siliguri | | Nilgiri | |
|---|---|---|---|---|---|---|---|---|
| | Infusion | Decoction | Infusion | Decoction | Infusion | Decoction | Infusion | Decoction |
| *Organic acids* | | | | | | | | |
| L-(+)Lactic acid | 68.68 ± 11.56 | 32.49 ± 9.73 | 11.68 ± 2.85 | 2.91 ± 4.49 | 19.71 ± 0.94 | 13.40 ± 2.04 | 12.19 ± 2.20 | 8.52 ± 0.80 |
| Fumaric acid | 0.37 ± 0.19 | 0.57 ± 0.20 | 0.19 ± 0.21 | 0.25 ± 0.21 | 0.21 ± 0.16 | tr | tr | tr |
| Gluconic acid | 0.66 ± 0.68 | 2.30 ± 2.76 | 2.26 ± 0.06 | tr | tr | tr | tr | tr |
| Glyceric acid | 17.50 ± 4.14 | 19.94 ± 5.39 | 7.77 ± 0.85 | 17.15 ± 4.10 | 14.61 ± 1.38 | 15.08 ± 0.71 | 24.88 ± 4.57 | 24.36 ± 2.64 |
| Glycolic acid | 5.92 ± 1.14 | 2.43 ± 0.91 | 1.81 ± 0.62 | 3.53 ± 0.87 | 2.14 ± 0.12 | 1.68 ± 0.16 | 4.94 ± 1.00 | 1.92 ± 0.19 |
| D-Malic acid | 37.51 ± 8.82 | 90.36 ± 31.62 | 39.87 ± 21.71 | 59.15 ± 32.95 | 132.71 ± 21.54 | 115.12 ± 21.57 | 68.56 ± 16.71 | 93.09 ± 13.09 |
| Malonic acid | 8.21 ± 1.62 | 5.26 ± 2.75 | 4.74 ± 0.52 | 2.63 ± 0.48 | 2.72 ± 0.86 | 1.36 ± 0.32 | 4.03 ± 1.10 | 2.29 ± 0.32 |
| Oxalic acid | 2.59 ± 0.96 | 0.61 ± 0.76 | 0.00 ± 0.00 | 8.08 ± 6.67 | 3.37 ± 2.59 | 2.16 ± 1.60 | tr | tr |
| Succinic acid | 39.53 ± 15.03 | 22.24 ± 7.73 | 15.93 ± 1.40 | 21.93 ± 2.14 | 25.49 ± 0.57 | 22.45 ± 0.38 | 35.39 ± 3.57 | 27.38 ± 1.31 |
| Gluconic acid lactone | tr | tr | tr | 3.07 ± 0.76 | tr | tr | tr | 388.87 ± 763.51 |
| *Inorganic acid* | | | | | | | | |
| Phosphoric acid | 49.84 ± 16.35 | 583.13 ± 354.38 | 148.83 ± 115.44 | 266.47 ± 51.82 | 153.61 ± 29.13 | 125.66 ± 34.09 | 37.44 ± 14.24 | 43.89 ± 9.66 |
| *Amino acids* | | | | | | | | |
| L-Alanine | 0.77 ± 0.78 | tr | 2.36 ± 0.62 | 1.76 ± 1.03 | 0.51 ± 1.03 | 2.02 ± 1.62 | tr | tr |
| Beta-alanine | 0.15 ± 0.10 | 0.38 ± 0.26 | 0.31 ± 0.62 | tr | tr | tr | 0.31 ± 0.23 | tr |
| L-Allothreonine | 1.25 ± 1.50 | 2.98 ± 1.05 | 3.41 ± 1.05 | tr | 2.80 ± 3.27 | 1.51 ± 3.02 | tr | tr |
| L-Asparagine | tr | tr | 5.95 ± 1.04 | 6.45 ± 1.64 | 17.70 ± 3.60 | 19.48 ± 1.67 | tr | tr |
| Aspartic acid | tr | 2.82 ± 1.14 | 7.64 ± 1.55 | 30.30 ± 41.57 | 9.35 ± 1.18 | 14.25 ± 10.32 | tr | tr |
| Glutamic acid | tr | 1.02 ± 2.04 | 1.73 ± 3.47 | 27.25 ± 11.33 | 18.22 ± 2.28 | 22.89 ± 2.04 | tr | tr |
| L-Glutamine | tr | tr | 2.20 ± 1.14 | 4.18 ± 1.54 | 1.56 ± 0.10 | 1.70 ± 1.13 | tr | tr |
| L-Leucine | 3.65 ± 0.70 | 1.58 ± 0.56 | 2.71 ± 0.20 | 5.81 ± 1.13 | 1.32 ± 0.88 | 0.77 ± 0.90 | tr | tr |
| DL-Isoleucine | 8.77 ± 1.58 | 3.99 ± 1.01 | 5.95 ± 0.42 | 11.48 ± 3.62 | 3.70 ± 1.61 | 3.52 ± 1.47 | tr | tr |
| L-Proline | 0.40 ± 0.40 | 0.14 ± 0.29 | 0.15 ± 0.29 | 0.91 ± 1.06 | tr | 0.40 ± 0.79 | tr | tr |
| L-Pyroglutamic acid | 123.55 ± 42.72 | 146.79 ± 69.97 | 120.83 ± 24.72 | 642.04 ± 68.31 | 75.53 ± 14.28 | 77.49 ± 12.23 | 42.89 ± 6.41 | 32.12 ± 2.91 |
| L-Serine | 0.81 ± 0.48 | 2.15 ± 0.77 | 3.60 ± 0.70 | 11.48 ± 2.31 | 5.40 ± 0.76 | 5.88 ± 0.79 | tr | tr |
| L-Threonine | 1.73 ± 1.36 | tr | tr | 8.31 ± 5.78 | 5.13 ± 0.90 | 4.90 ± 3.27 | tr | tr |
| L-Tryptophan | 6.09 ± 3.99 | 2.91 ± 5.81 | 14.86 ± 4.17 | 20.50 ± 1.91 | tr | 7.70 ± 1.09 | tr | tr |
| L-Tyrosine | 27.61 ± 4.15 | 21.73 ± 6.31 | 43.47 ± 7.51 | 71.82 ± 10.99 | 6.82 ± 1.37 | 8.87 ± 0.42 | 4.20 ± 4.87 | 4.14 ± 4.83 |
| L-Valine | 20.98 ± 4.54 | 9.79 ± 3.68 | 13.11 ± 1.39 | 24.66 ± 6.09 | 11.82 ± 0.84 | 11.93 ± 4.00 | 0.71 ± 0.35 | 0.46 ± 0.08 |

Table 1 (*Continued*)

| Metabolites | Doors | | Guahati | | Siliguri | | Nilgiri | |
|---|---|---|---|---|---|---|---|---|
| | Infusion | Decoction | Infusion | Decoction | Infusion | Decoction | Infusion | Decoction |
| *Sugar alcohols* | | | | | | | | |
| Arabitol | tr | 3.38 ± 4.26 | 2.87 ± 1.89 | 8.22 ± 2.29 | tr | tr | tr | tr |
| Glycerol | 486.52 ± 251.43 | 863.78 ± 234.19 | 145.76 ± 20.03 | 249.33 ± 110.46 | 81.66 ± 12.60 | 89.34 ± 31.07 | 504.11 ± 54.64 | 526.95 ± 33.76 |
| Glycerol 1-phosphate | 4.56 ± 1.09 | 16.09 ± 5.80 | 6.30 ± 0.50 | 18.05 ± 2.92 | 3.52 ± 0.46 | 4.64 ± 0.22 | 4.66 ± 1.71 | 4.03 ± 0.40 |
| D-Threitol | 0.50 ± 0.55 | 14.22 ± 27.88 | 0.83 ± 0.21 | 4.64 ± 6.46 | 4.16 ± 5.84 | 3.85 ± 5.41 | 0.26 ± 0.20 | 37.51 ± 35.28 |
| D-Mannitol | 7.35 ± 3.43 | 9.52 ± 2.51 | 6.74 ± 1.18 | 8.52 ± 1.75 | 6.36 ± 0.42 | 3.98 ± 4.08 | 4.75 ± 0.54 | 4.58 ± 2.12 |
| *Sugars* | | | | | | | | |
| Sucrose | tr | 1049.03 ± 774.86 | 466.86 ± 932.48 | 2445.48 ± 1634.37 | 2755.59 ± 2041.10 | 5280.73 ± 3549.56 | 732.48 ± 1464.95 | 3263.53 ± 133.55 |
| Raffinose | 15.29 ± 5.74 | 19.55 ± 5.34 | 28.08 ± 5.97 | tr | tr | tr | 15.72 ± 10.60 | 14.13 ± 9.44 |
| *Phenols* | | | | | | | | |
| *O*-Acetylsalicylic acid | 1.93 ± 1.26 | tr | 1.32 ± 1.09 | 4.90 ± 4.40 | 3.82 ± 0.70 | 6.16 ± 3.55 | 1.63 ± 1.26 | tr |
| Arbutin | 2.11 ± 1.21 | 0.55 ± 0.64 | 1.17 ± 0.89 | | 1.48 ± 0.85 | tr | tr | 1.28 ± 0.69 |
| Caffeic acid | 0.52 ± 0.07 | 0.40 ± 0.34 | 0.25 ± 0.08 | 0.37 ± 0.27 | tr | tr | tr | tr |
| Catechin | 5.40 ± 4.82 | 9.43 ± 7.76 | 9.71 ± 6.53 | 43.75 ± 16.68 | 12.16 ± 0.30 | 27.59 ± 18.44 | 4.14 ± 0.68 | 4.31 ± 0.09 |
| Chlorogenic acid | 6.10 ± 0.33 | 10.71 ± 8.00 | 4.84 ± 0.28 | 13.95 ± 2.22 | 7.69 ± 0.48 | 13.84 ± 0.86 | 5.02 ± 1.07 | 4.30 ± 0.20 |
| Epicatechin | 1.97 ± 0.33 | 1.60 ± 0.41 | 3.02 ± 0.60 | 7.49 ± 1.09 | 1.02 ± 0.32 | 2.97 ± 0.08 | 2.59 ± 0.21 | 2.80 ± 0.08 |
| (−)-Epicatechin | 12.28 ± 3.49 | 29.03 ± 14.00 | 33.52 ± 5.01 | 220.42 ± 143.50 | 39.87 ± 1.66 | 168.15 ± 91.11 | 19.80 ± 1.45 | 24.06 ± 1.14 |
| Epigallocatechin | 87.69 ± 26.10 | 123.68 ± 62.68 | 57.31 ± 6.47 | 275.26 ± 186.45 | 56.87 ± 2.92 | 224.58 ± 184.49 | 60.39 ± 3.86 | 66.34 ± 2.23 |
| Gallic acid | 1320.15 ± 174.24 | 1756.13 ± 507.26 | 1621.10 ± 803.53 | 1780.27 ± 554.01 | 1959.65 ± 146.45 | 2753.12 ± 370.97 | 1944.48 ± 295.29 | 1623.29 ± 2048.25 |
| 4-Hydroxybenzoic acid | tr | tr | tr | 0.61 ± 0.44 | tr | tr | 0.61 ± 0.46 | 0.63 ± 0.06 |
| 3,4-Dihydroxybenoic acid | 22.65 ± 23.19 | 27.81 ± 22.03 | 6.74 ± 7.83 | 30.10 ± 22.20 | 6.63 ± 2.01 | 5.84 ± 3.96 | 41.95 ± 4.50 | 40.17 ± 5.82 |
| Phloroglucinol | 1.06 ± 0.15 | 3.15 ± 0.48 | 4.24 ± 7.11 | 5.06 ± 4.44 | 0.49 ± 0.21 | 3.36 ± 0.90 | 1.45 ± 0.17 | 4.40 ± 2.94 |
| Pyrogallol | 0.23 ± 0.39 | 0.79 ± 1.00 | tr | 2.26 ± 3.70 | 0.28 ± 0.37 | 0.40 ± 0.79 | tr | tr |
| Quinic acid | 1518.37 ± 487.66 | 3193.93 ± 2541.39 | 3018.06 ± 2413.46 | 2145.28 ± 513.79 | 2734.01 ± 2143.35 | 4713.67 ± 3862.05 | 3764.45 ± 1895.25 | 1515.46 ± 776.09 |
| Shikimic acid | 551.79 ± 44.49 | 527.83 ± 159.88 | 578.36 ± 54.13 | 1096.42 ± 143.20 | 530.10 ± 16.82 | 633.49 ± 92.33 | 409.00 ± 33.89 | 283.96 ± 20.31 |
| *Fatty acids* | | | | | | | | |
| Palmitic acid | 5.43 ± 1.92 | 10.94 ± 4.53 | 13.17 ± 6.46 | 4.70 ± 1.04 | 3.52 ± 0.82 | 2.90 ± 0.64 | 2.35 ± 0.65 | 2.18 ± 0.28 |
| Stearic acid | 6.14 ± 2.09 | 19.41 ± 8.15 | 22.11 ± 14.68 | tr | tr | 1.37 ± 2.73 | 4.32 ± 0.60 | 3.27 ± 0.82 |

tr, trace.

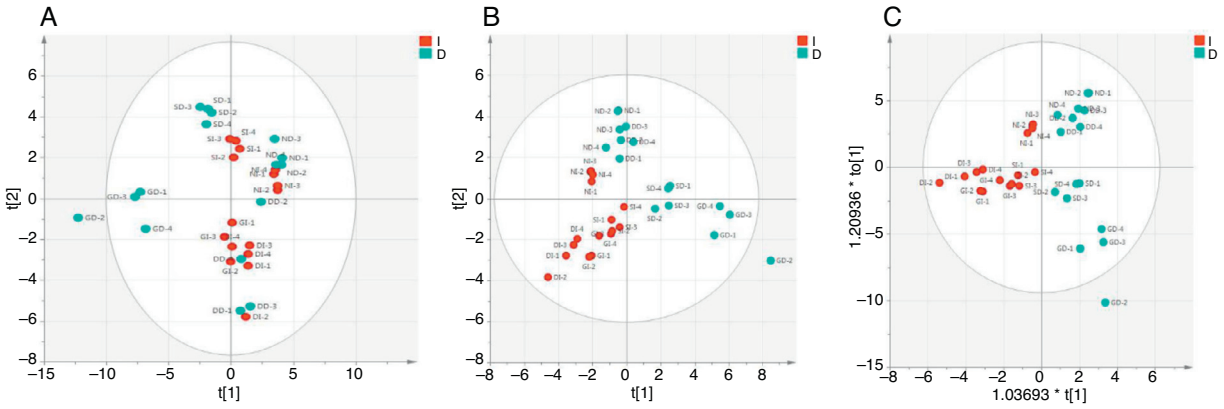


**Fig. 1.** Multivariate analyses segregating BT infusions and decoctions on the basis of metabolites. (A) PCA score plot; (B) PLS-DA score plot; (C) OPLS-DA score plot. I, infusion; D, decoction; DD, doors decoction; DI, doors infusion; GD, Guwahati decoction; GI, Guwahati infusion; ND, Nilgiri decoction; NI, Nilgiri infusion; SD, Siliguri decoction; SI, Siliguri infusion.

BT extracts analyzed by HPLC (Ray et al., 2014). During the present work the metabolite profile of infusion and decoction of four samples of BT were compared using GC–MS based metabolomics approach to identify more compounds present in tea extracts. The metabolomics technology helped to identify fifty one components including ten organic acids, one inorganic acid, sixteen amino acids, two sugars and five sugar alcohols, fifteen phenols and flavonoids, two fatty acids (Table 1). Present analysis detected phenols like *O*-acetylsalicylic acid, arbutin, caffeic acid, 4-hydroxybenzoic acid, phloroglucinol, quinic acid, shikimic acid and some sugars which were not reported earlier, to the best of our knowledge. However, some compounds *e.g.* different theaflavins identified earlier by HPLC method (Ray et al., 2014) could not be identified by the method used during the present library assisted GC–MS analysis. The data presented in Table 1 are normalized relative response ratios calculated by dividing peak areas of the metabolites by sample weight and by peak area of the internal standard. The data were analyzed by different multivariate analyses PCA, PLS-DA and OPLS-DA. PLS-DA and OPLS-DA (Fig. 1a–c) models showed good segregation among groups, decoction and infusion, based on metabolites. The findings suggest that the tea samples were different on the basis of identified metabolites.

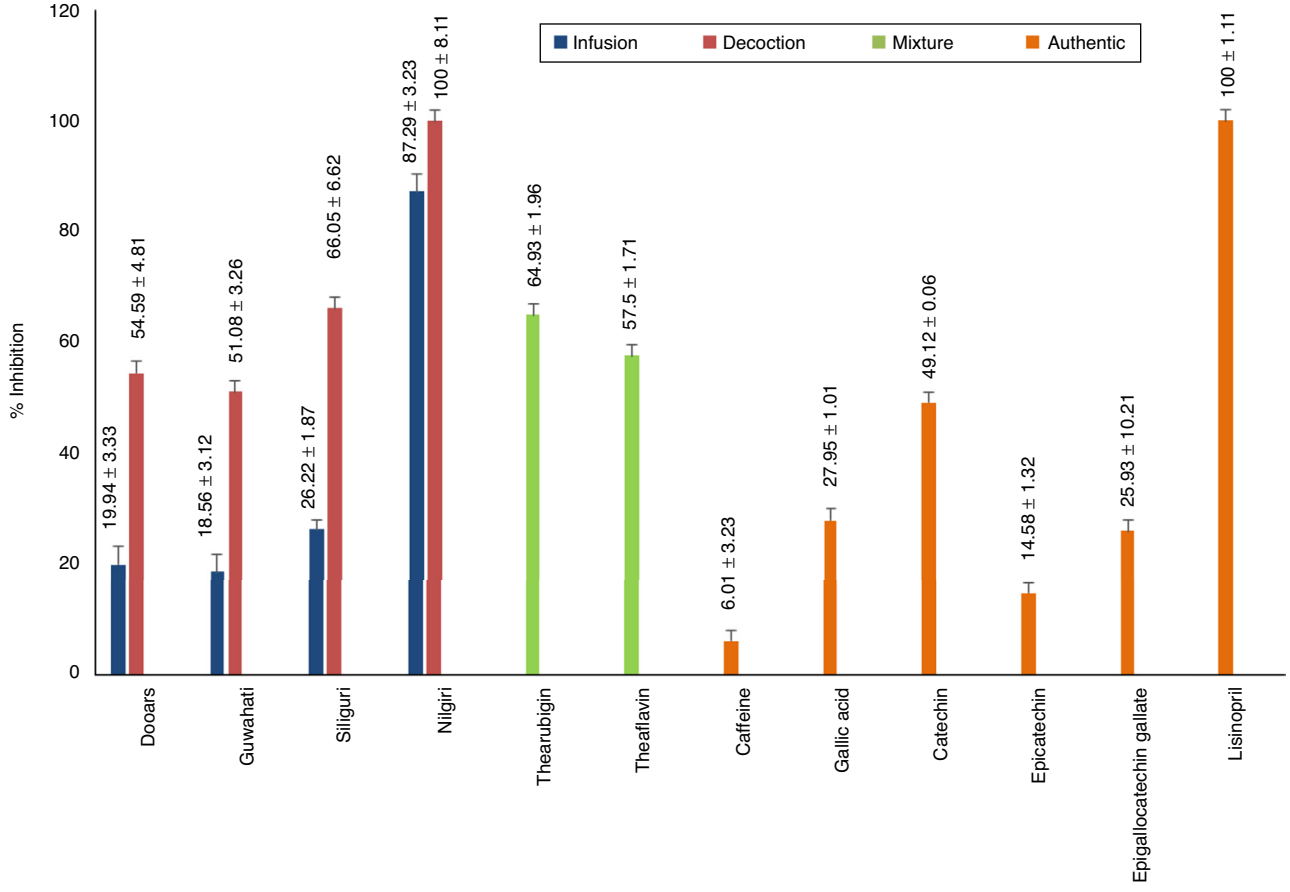


**Fig. 2.** Angiotensin converting enzyme inhibition by BT (infusions and decoctions), theaflavin, and thearubigin at 15 μg/ml; authentic samples at 37 μM concentration.

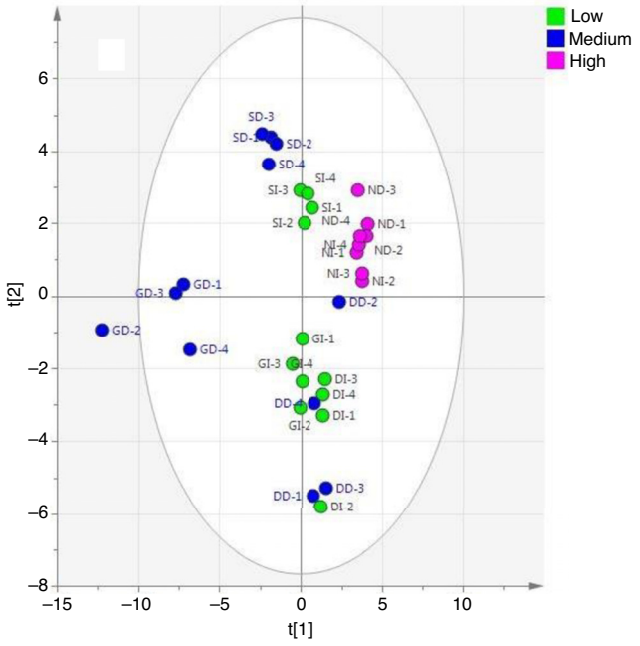


**Fig. 3.** PCA score plot separating BT infusions and decoctions on the basis of ACE inhibition activity. DD, doors decoction; DI, doors infusion; GD, Guwahati decoction; GI, Guwahati infusion; ND, Nilgiri decoction; NI, Nilgiri infusion; SD, Siliguri decoction; SI, Siliguri infusion.

*Angiotensin converting enzyme inhibitory activity*

ACE inhibitory activity of BT infusions, BT decoctions were compared at 15 μg/ml concentration. Among all the tea samples, Nilgiri infusion and decoction exhibited highest activities (87% inhibition by infusion and 100% inhibition by decoction). Lisinopril (37 μM) exhibited 100% inhibition. But other tea extracts had activity lower than that of Lisinopril (Fig. 2). Nilgiri samples showed no significant differences in activity between infusion and decoctions. However, in the other three samples, activities of decoctions were higher than the infusions. PCA (Fig. 3) model showed good classification among groups based on activity. This suggested that the ACE inhibitory activity of BT infusions and decoctions were different. A few identified metabolites, available in the laboratory, were tested for the ACE inhibitory properties at 37 μM concentration. Thearubigin is a complex mixture (Kuhnert, 2010). Procured theaflavin was also a mixture of theaflavin and theaflavingallates. So the activities of these two compounds were tested at 15 μg/ml concentration. Thearubigin, and theaflavin inhibited the enzyme by more than 50%. None of the authentic phenols and flavonoids inhibited the enzyme more than 50%. Amongst the authentic compounds, catechin showed highest activity. The alkaloid caffeine also inhibited the enzyme, although the activity of this compound was found to be lowest (Fig. 2). Lisinopril (37 μM), one of the most widely used ACE inhibitor (Natesh et al., 2003), inhibited 100% enzyme activity. Previously, extracts of green tea, black tea, rooibos tea and also the main flavanols and purine alkaloids in green and black tea were examined for their effects on ACE in cultured endothelial cells from human umbilical veins (HUVEC). Green tea, black tea and rooibos tea exhibited dose-dependent inhibition of ACE activity in HUVEC. (−)-Epicatechin, (−)-epigallocatechin, (−)-epicatechingallate and (−)-epigallocatechingallate also inhibited ACE activity in HUVEC (Persson et al., 2006). The present study shows that thearubigin, theaflavin, catechin had significantly higher activity than those of epicatechin and epigallocatechin gallate. Thus the present study suggests that BT may have beneficial effect in lowering blood pressure because of ACE inhibitory properties.

Although not calculated, it is obvious that the quantity of each of these components were much lower in the crude extract of tea at this concentration (15 μg/ml). Thus, from the above chemometric findings and the comparison of activities of tea extracts and some flavonoids and caffeine, it could be inferred that the tea components have either additive or synergistic activities for inhibiting ACE. Such synergistic activities have recently been published (Colon and Nerin, 2016) for DPPH radical scavenging activities in green tea.

## Conclusion

Infusions and decoctions obtained from four different samples of black tea were analyzed for metabolites and ACE inhibition properties. The infusions and the decoctions were found to be different on the basis of metabolite level. All the extracts inhibited the enzyme ACE. Nilgiri decoction showed activity similar to that of the drug Lisinopril. Of the fifty one different metabolites identified by GC–MS, thearubigin, theaflavin, catechin, gallic acid, epicatechin and epigallocatechin gallate, caffeine inhibited ACE the first three showing higher activity than the others.

## Authors' contributions

SR performed experiments. MD and KC did statistical analysis and interpretation of results. BD provided idea, project plan. All the authors were involved in the preparation of the manuscript.

## Conflicts of interest

The authors declare no conflicts of interest.

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