# American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS American Journal of Food Technology

ISSN 1557-4571
DOI: 10.3923/ajft.2017.358.366

# Research Article
# Pressurized Liquid Extraction of Antioxidant Compounds from Green Tea

Swapnil Ganesh Jaiswal and Satyanarayan Naik

Supercritical Fluid Extraction Laboratory, Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi-110016, India

## Abstract

**Background and Objective:** India is the second largest producer of green tea. The green tea is a major source of health promoting antioxidant group (catechins). The main aim of the present study was to extract green tea antioxidant rich extract by using pressurized extraction technique for antioxidant study. **Materials and Methods:** Four varieties of green tea were extracted by using pressurized liquid extraction (PLE) method using ethanol as the solvent. In order to get maximum yield of biologically active compounds, extraction conditions were optimized in terms of pressure, temperature and solvent flow rate. Results of total phenol, flavonoids and antioxidant extraction were analyzed statistically by using one way ANOVA with Tukeys B-test. **Results:** The optimum pressure for recovering maximum phenolic acids and major catechins was found to be about 150 bar. Out of seven investigated phenolic acids and five catechins, vanillin followed by gallic acid, catechin and epigallo catechin gallate were found to be in major concentrations. Total phenol content of all four varieties was found to be more than total flavonoid content. Darjileeng variety gave best results for total phenol content (607.03 mg GAE $g^{-1}$ dry weight basis) and percent DPPH inhibition (74.40%) while Assam and Nilgiri variety gave best results for total flavonoid content (79.15 mg QE $g^{-1}$ dry weight basis) and percent ABTS inhibition (51.34%). Antimicrobial activity (bacterial and fungal) of extracts from all four varieties was screened through well diffusion method in order to study their inhibitory effect. Bacterial strains were found to be more sensitive than fungal strains. **Conclusion:** The PLE could be the promising method for extraction of antioxidant compounds (phenolic and flavonoids) from green tea.

Key words: Pressurized liquid extraction (PLE), green tea (*Camellia sinensis*), polyphenols, catechins, antioxidant, antimicrobial

Citation: Swapnil Ganesh Jaiswal and Satyanarayan Naik, 2017. Pressurized liquid extraction of antioxidant compounds from green tea. Am. J. Food Technol., 12: 358-366.

Corresponding Author: Swapnil Ganesh Jaiswal, Supercritical Fluid Extraction Laboratory, Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi-110016, India Tel: +91-8587035936



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

India is the second largest producer of green tea and it is the most popular beverage consumed widely among all sections of people. Assam, Himachal Pradesh, West Bengal, Tamil Nadu, Kerala and Karnataka are the major tea producing states in India. Green tea is one of the non-fermented type tea among four types (white tea, black tea and oolong tea). Due to least processing unit operations, green tea conserve most of the antioxidant compounds responsible for taste, flavour and several health benefits[1,2]. Flavanols caffeine and flavanols are the chemically active compounds present in green tea which is responsible for antioxidant activities[3]. Green tea polyphenols have special flavanol group antioxidant compounds called as catechins. These green tea catechins have wide applications in the area of food processing and preservation by protecting lipid based food materials from its oxidation and enhance its shelf life[4]. Apart from antioxidant properties, other medicinal properties like anti-microbial, anti-malarial, anti-cancerous and other health protecting activities of green tea active compounds were described through different studies done by earlier researchers[5-7]. According to the previous study carried out on extraction of tea leaves for catechins, it is confirmed that first four apical leaves contains high quality catechins than lower old tea leaves[8]. Therefore, production of green tea from apical tea leaves improves its antioxidant potential and exhibits itself as a healthier green tea. Extraction of such a biologically active compounds from the leafy matrices of tea using green extraction technique fostered new horizon in the applied area of food and pharmaceutical industries. There are three main reasons behind the use of green extraction techniques as it takes less time, less solvents and require less energy than solvent extraction[9]. Several other extraction techniques namely cold water extraction, hot water extraction, solvent extraction, pressurized solvent extraction, reflux extraction, supercritical fluid extraction, ultrasound assisted extraction, microwave assisted extraction were adopted by different researchers in their studies[10-14]. Recently, new green solvent extraction technique was also introduced which is based on the use of Deep Eutectic Solvents (DESs) for extraction of catechins from green tea[15]. Extraction solvents, extraction time and extraction temperature are the key factors which affect the extraction yield of target oriented compounds from green tea leaves. In a different study it was observed that increasing heating action during extraction adversely affect on the chemical composition of green tea, where decreased concertation of total catechins were noted at a temperature range between 85-120°C[16]. Most of the green tea extraction study confirms the polar nature of investigated green tea active compounds catechins and caffeine where polar solvents like water, ethanol, methanol and acetonitrile were used for extraction.

The main aim of this study was to extract biologically active compounds from green tea by using green extraction technique like PLE. In order to get better yield of extract and polyphenols, process parameter like pressure was optimized by keeping constant temperature and flow rate. Group of antioxidant compounds such as phenolic acids and catechins were quantified by using high performance liquid chromatography (HPLC). Antioxidant and antimicrobial study of extracts was done by performing different assay like TPC, TFC, DPPH, ABTS and well diffusion method.

## MATERIALS AND METHODS

**Materials and extraction procedures:** Green tea leaves of four different varieties namely Kangra, Assam, Darjileeng and Nilgiri were collected from four different states of India viz. Himachal Pradesh, Assam, West Bengal and Tamil Nadu, respectively during end of the winter season i.e., 1st week of March, 2017. Extraction studies in the PLE method were conducted at different pressures varying from 50-200 bar, keeping 3 mL $min^{-1}$ constant solvent (ethanol) flow rate. All the experiments were carried out in PLE laboratory set up (Fig. 1) consisting of extractor, sample collector, water bath supercritical pump (SFT10, USA), metering pump for solvent (Eldex, NAPA, USA). About 30 g sample of ground tea leaves was employed in 500 mL extracting vessel for each run. Percent extraction yield was calculated after concentrating extract in rotary evaporator (Ika, India).

**Chemical reagents:** Analytical grade solvents namely ethanol, methanol were purchased from Merck (Darmstadt, Germany). Reagents, Folin-Ciocalteu phenol reagent, 1, 1-diphenyl-2-picryhydrazyl radical (DPPH), 2, 4, 6-Tri-(2-pyridyl)-striozine (TPTZ) was from Sigma-Aldrich (St. Lous. MO). Sodium carbonate and potassium per sulphate was purchased from CDH fine chemicals (New Delhi, India). The HPLC grade chemical standards namely gallic acid, vanillin, myricetin, rutin, quercetin, kaempherol and protocatechuic acid were procured from sigma chemicals. Standard catechin Kit (EC, EGC, ECG and EGCG) was obtained from ChromaDex (Muirlands, CA). Water used for analysis was of distilled grade.

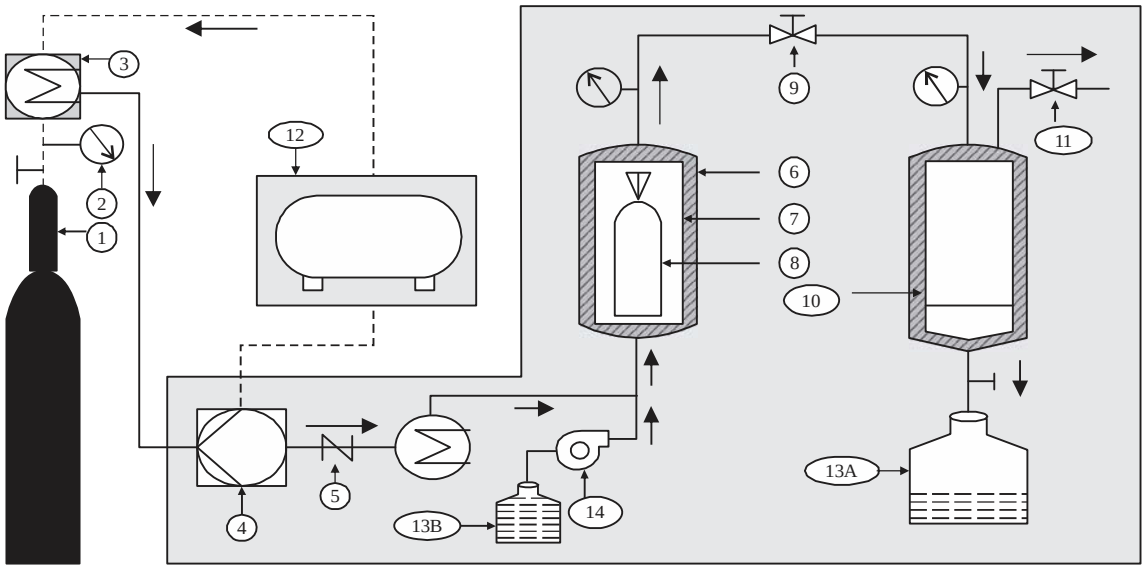


Fig. 1: Process flow chart for PLE Unit

1: $CO_2$ cylinder, 2: Flow meter, 3: Heat exchanger, 4: $CO_2$ pump, 5: Non-returning valve, 6: Cooling/Heating jacket, 7: Extractor, 8: Raw material bag, 9: Automated Back Pressure Regulator (ABPR), 10: Product collector, 11: Manual ABPR, 12: Chiller, 13A: Sampling bottle, 13B: Solvent bottle, 14: Solvent dosing pump

**HPLC analysis for phenolics and catechins:** Quantitative determination of extracts were done in HPLC (Waters 600 HPLC system) with photodiode array (PDA) detector (Waters 2998). All standards and samples were prepared in HPLC grade methanol. Samples were filtered through 0.45 micron filter before injection to HPLC. For both phenols and catechins quantification reverse phase Kinetic C18 column (2.1X100 mm ID) was used. Wavelength of PDA for both phenols and catechins was set at 215 and 280 nm, respectively. A mixture of isocratic solvent system containing 40% methanol, 15% acetonitrile and 45% water of 1% acetic acid was pumped with 0.5 mL $min^{-1}$ flow rate for 20 min to separate peaks of seven phenolic acids. Binary system of solvent was used for catechins where water (A) containing 0.1% acetic acid and acetonitrile (B) containing 0.1% acetic acid pumped with 1 mL $min^{-1}$ flow rate for 20 min. Gradient program of binary mobile phase was set as: 0-15% B for 2 min 15-35% B for 1 min, 35-90% B for 1 min, 90-15% for 1 min and 15-20% for 15. Results of extracts on dry weight (dw) basis are expressed in mg $g^{-1}$and mg $kg^{-1}$ for catechins and phenolic acids, respectively.

**Total phenols and total flavonoids:** Total phenol content (TPC) of extracts was measured by standard Folin-Ciocalteu method with slight modification in the preparation of sample/standard as described earlier[17]. Instead of using 200 µL sample[17], 100 µL sample extract was added in 1 mL Folin-Ciocalteu reagent (diluted up to 10 fold). After 8 min incubation, 3 mL sodium carbonate (7.5%) was added and the mixture was incubated for 1 h at room temperature. All extract samples and standards were prepared in methanol. All readings at 765 nm was recorded in UV-Visible spectrophotometer (Shimadzu, UV-2600 Kyoto, Japan). Results were expressed in milligram gallic acid equivalent per gram (mg GAE $g^{-1}$).

Total flavonoid content (TFC) of extracts was determined by the method of Hosu *et al.*[18] with slight modification in the used standard. Instead of using standard rutin[18], quercetin was used to prepare flavonoid standard curve and express the results in milligram quercetin equivalent (QE) per gram (mg QE $g^{-1}$). A mixture of 400 µL of $AlCl_3$ (25g $L^{-1}$), 500 µL of sodium acetate (100 g $L^{-1}$) and 4 mL distilled water was added in 500 µL of extracts/standards. After 15 min, the absorbance of the mixture was measured in spectrophotometer at 415 nm. Quercetin standard concentration curve for flavonoids was obtained in the range of 0-100 µg $mL^{-1}$.

**Antioxidant study:** The DPPH radical scavenging activity of extracts was measured by using the method of Patel *et al.*[17]. A reaction mixture of 100 µL extracts and 3900 µL DPPH solution (0.004%) was prepared and incubate it in dark for 60 min. Readings were recorded on spectrophotometer at 515 nm against blank methanol.

Th ABTS radical scavenging activity of extracts was measured by the used method of Jaiswal *et al.*[19]. The ABTS stock solution was prepared by adding 7 mM ABTS and

2.45 mM potassium persulphate in equal quantities and allowed this reaction mixture for 16 h incubation in dark. Working stock solution of 0.700±0.005 absorbance at 734 nm was prepared by diluting reaction mixture with 80% ethanol. 3900 µL ABTS working stock solution was added in test tube containing 100 µL extracts and the mixture was allowed to incubate for 5 min to measure absorbance. Both DPPH and ABTS radical scavenging activity results of extracts were measured in terms of percent inhibition (Inhibition %).

**Antimicrobial study:** All bacterial and fungi used in the present study were procured from ATCC [*Aspergillus brasiliensis* (ATCC16404), *Salmonella abony* (ATCC 6017), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) and *Candida albicans* (ATCC 10231)]. All bacterial and fungal strains were routenly subcultured and maintained on nutrient agar (NA) and potato dextrose agar (PDA), respectively throughout the experiment. For each experiement, microorganisms were freshly subcultured and used.

Well diffusion method of antimicrobial assay was employed for the present study. Bacterial (OD 0.45 at A610 nm) and fungal ($10^8$ conidia $mL^{-1}$) suspensions were prepared in sterile distilled water. About 50 µL of bacterial and fungal suspensions were spread plated on NA and PDA, respectively. Wells were bored at four corners of each plate using sterile cork borrer (6 mm). Each well was filled with 100 µL of test extracts (10 mg $mL^{-1}$concentration). Further plates with NA were incubated at 35°C for 36 h and plates with PDA were incubated at 28°C for 5 days. Towards the end of incubation period the zone of inhibition was measured in mm and tabulted. For each test microorganism and extract the experiments were reapeated thrice with 3 replications for each experiment.

**Statistical analysis:** Statistical software SPSS windows version 16.0 (SPSS Corporation, Chicago, IL) was used for analysis. All the results of triplicate determinations were analyzed through one way ANOVA with Tukeys B-test[17]. Reported results in tables are the values of Mean±SD. Means were accepted as significantly different at 95% confidence interval ($p<0.05$).

## RESULTS AND DISCUSSION

**Pressurized liquid extraction of green tea:** The extraction process for green tea antioxidants was optimized by varying pressure from 50-200 bar with constant 3 mL $min^{-1}$ flow rate of extraction solvent (ethanol). Jacket temperature of extraction vessel was maintained at 75±1°C. Sampling of extracts were done in batches after each 15 min intervals. Total extraction time for each experiments was set for 60 min. All the extracts obtained after each experiment was concentrated in rotary evaporator and percent yield of oleoresin was calculated. Results of this study show that as the pressure increases from 50-200 bar, yield of oleoresin increases while after 150 bar there was no significant difference found in percent yield (Fig. 2). About 150 bar pressure was found to be the optimized pressure for green tea extract of Darjileeng variety. All the four varieties of green tea were extracted with 150 bar pressure and 3 mL $min^{-1}$ solvent flow rate. Percent yield of extracts for all four varieties Kangra, Nilgiri, Assam, Darjileeng was found to be 19.93±1.02, 21.12±0.50, 18.13±1.08, 23.48±0.53, respectively. Values of total phenol content as shown in Fig. 2 were also found to be directly proportional with percent extraction yield.

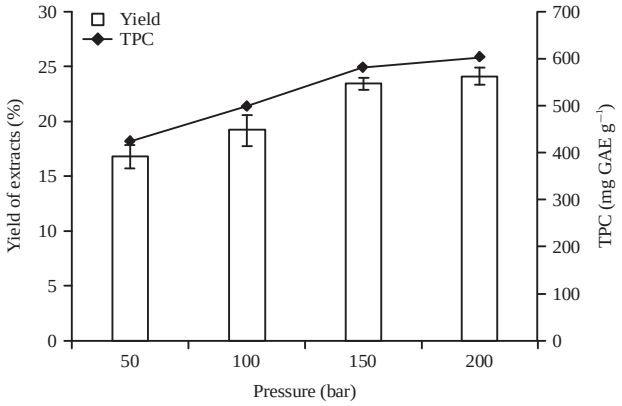


Fig. 2: Effect of increasing pressure on the yield of green tea extract of Darjileeng variety and total polyphenol content (TPC)
Values are the Mean±SD of triplicate assays

**HPLC analysis of phenolic acids and major catechins:** The concentrations of the major catechins (EGC, C, EC, EGCG and ECG) as well as seven phenolic acids namely gallic acid, vanillin, myricetin, rutin, protocatechuic acid, quercetin and kaempherol were investigated by using HPLC (Fig. 3). Concentrations of individual catechin compound and phenolic acid in the green tea extracts of the four varieties are given in Table 1. In phenolic acids, rutin, kaempherol, quercetin and protocatechuic acid was not detected. Vanillin was found to be a predominant phenolic acid followed gallic acid and myricetin. Green tea extracts of Nilgiri tea contain highest amount of vanillin (3.40 mg $kg^{-1}$ dw) while gallic acid (1.30 mg $kg^{-1}$ dw) was highest in Assam tea. In relation to flavanols, catechin and epigallocatechin gallate are the

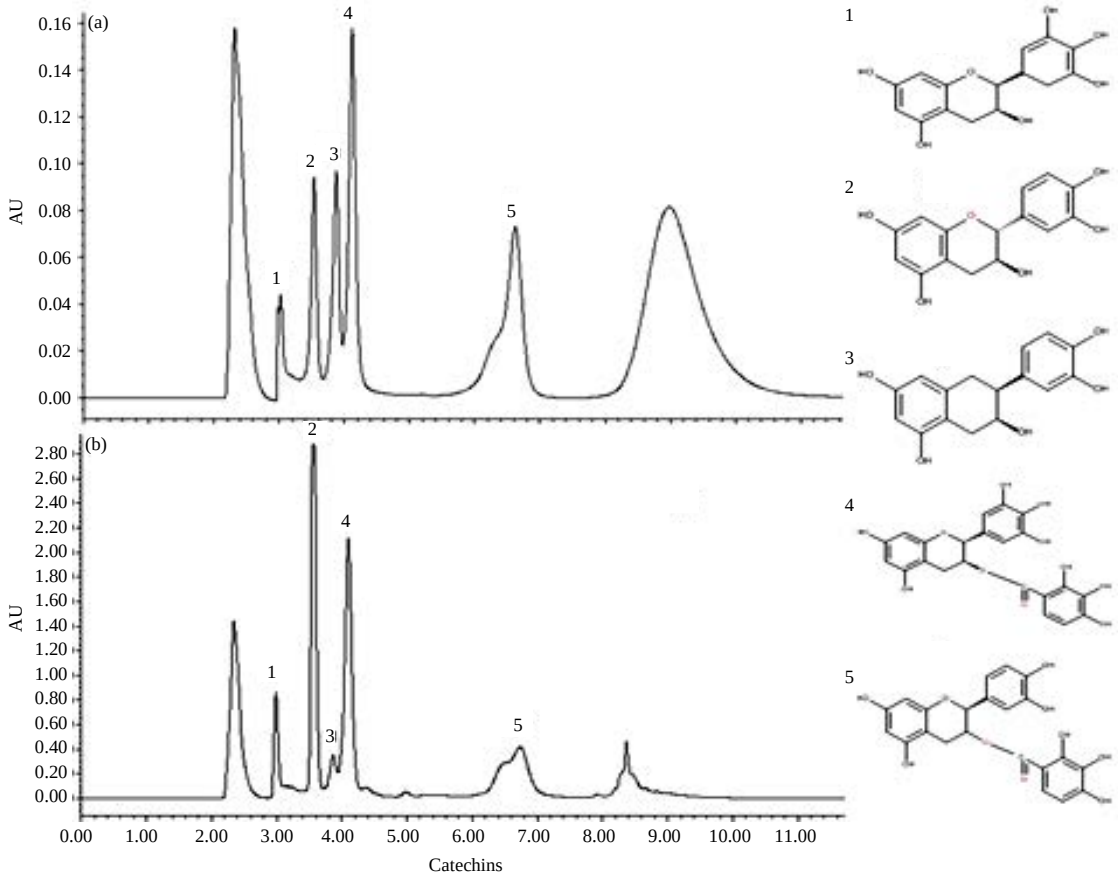


Fig. 3: Group of major catechin compounds investigated for antioxidant activities (a) Standard, (b) Green tea extracts
1: Epigallocatechin (EGC), 2: Catecin (C), 3: Epicatechin (EC), 4: Epigallocatechin gallate (EGCG), 5: Epicatechingallate (ECG)

Table 1: Quantitative analysis of catechins and phenolic acids by using HPLC

| Active constituent in green tea extract | Green tea variety | | | |
|---|---|---|---|---|
| | Kangra | Nilgiri | Assam | Darjileeng |
| **Catechins (mg g$^{-1}$ dw)** | | | | |
| EGC | 11.77 | 9.54 | 10.81 | 12.06 |
| Catechin | 53.81 | 55.33 | 33.83 | 57.82 |
| EC | 4.95 | 4.21 | 4.94 | 3.64 |
| EGCG | 26.92 | 24.75 | 28.32 | 30.47 |
| ECG | 4.85 | 4.78 | 4.24 | 8.00 |
| **Phenolic acids (mg kg$^{-1}$ dw)** | | | | |
| Gallic acid | 1.07 | 1.06 | 1.30 | 1.09 |
| Vanillin | 3.09 | 3.40 | 2.41 | 2.10 |
| Myricetin | 0.1 | 0.08 | 0.02 | nd |
| Rutin | nd | 0.01 | nd | nd |
| Protocatechuic acid | nd | nd | nd | nd |
| Quercetin | nd | nd | nd | nd |
| Kaempherol | nd | nd | nd | nd |

All values are the Mean±SD, n = 3, nd: Not detected

dominant catechins in all four variety extracts ranging from 33.83-57.82 and 24.75-30.47 mg g$^{-1}$ dw, respectively. This results supported the previously reported findings of Balentine *et al.*[20] and Rusak *et al.*[21], who analyzed the detailed composition of catechins and phenolic acids. Total catechin concentration in green tea extracts of the four varieties was observed in the range of 82.14-111.99 mg g$^{-1}$ dw. The four varieties in descending order of total catechin concentration in their extractsis Darjileeng>Kangra>Nilgiri>Assam. Bronner and Beecher[22] reported that concentration values of major catechins are not same in all the HPLC due to some factors like changing mobile phase, varying extraction or reaction conditions (time, temperature and pressure) and extraction solvents/substrates.

**TPC, TFC and antioxidant activity of green tea extracts:** The TPC, TFC, DPPH and ABTS assay of all four varieties of green tea extracts is reported in Table 2. Superscript letters of result reported in column for all four varieties are significantly different with $p<0.05$. Values of TPC and TFC varied widely among selected four tea variety. The TPC and TFC of all extracts ranged from 309.06-607.03 mg GAE g$^{-1}$ dw and 25.30-79.15 mg QE g$^{-1}$ dw, respectively. The TPC of Darjileeng tea followed by Kangra and Nilgiri was observe to be highest

Table 2: Total phenol, total flavonoid and total antioxidant activities of four varieties of green tea extracts

| Green tea variety | TPC (mg GAE $g^{-1}$ dw) | TFC (mg QE $g^{-1}$ dw) | Total antioxidant activity | |
|---|---|---|---|---|
| | | | DPPH (% inhibition) | ABTS (% inhibition) |
| Kangra | 500.63±17.63$^{c}$ | 25.30±0.25$^{a}$ | 72.30±0.69$^{c}$ | 36.29±0.87$^{a}$ |
| Nilgiri | 401.81±18.08$^{b}$ | 45.05±0.07$^{c}$ | 59.63±0.67$^{a}$ | 51.34±1.10$^{c}$ |
| Assam | 309.06±55.82$^{a}$ | 79.15±0.92$^{d}$ | 69.63±0.50$^{b}$ | 43.12±0.90$^{b}$ |
| Darjileeng | 607.03±16.21$^{d}$ | 28.33±0.60$^{b}$ | 74.40±0.35$^{d}$ | 35.82±0.91$^{a}$ |

Different superscript letters in same columns are significantly different at $p<0.05$, Values are the Mean±SD of triplicate assays

Table 3: Antimicrobial activities of four varieties of green tea extracts against bacterial and fungal pathogens

| Green tea variety | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* | *S. abony* | *E. coli* | *A. flavous* | *A. brasiliensis* | *C. albicans* |
| Kangra | 6.5 | 5.5 | 8.5 | 0.0 | 0.0 | 0.0 |
| Nilgiri | 7.0 | 6.5 | 9.5 | 0.0 | 3.5 | 4.5 |
| Assam | 5.0 | 4.0 | 7.0 | 0.0 | 0.0 | 0.0 |
| Darjileeng | 7.5 | 6.0 | 12 | 3.0 | 3.0 | 4.0 |

while Assam variety gave lowest phenol content. The TFC was calculated against quercetin standard. In comparison to TPC value of TFC was found to be lower. Highest concentration of TFC among all the four varieties was observed for Assam (79.15 mg QE $g^{-1}$ dw) followed by Nilgiri (45.05 mg QE $g^{-1}$ dw). DPPH and ABTS assay was used to measure total antioxidant activity of all four variety extracts. Results were reported in terms of percent inhibition. The DPPH assay of percent inhibition for all varieties was found in the range of 59.63-74.40%. Highest percent inhibition was observed for Darjileeng (74.40%) followed by Kangra (72.30%). Trend of total phenol content results for selected four varieties was found to be directly proportional to the result of DPPH percent inhibition of all four varieties except Nilgiri (59.63%) which was observed to be lower than Assam (69.63%) variety. Percent ABTS activity was found in the range of 35.82-51.34%. Opposite trend of percent inhibition activity was observed compared to DPPH assay for four varieties. Nilgiri (51.34%) exhibited highest inhibition followed by Assam (43.12%). Lower percent inhibition activity was measured for both Kangra (36.29) and Assam 35.82) variety. Overall ABTS activity for four variety green tea extracts was observed to be lower than that of DPPH assay.

**Antimicrobial activity of green tea extracts:** The inhibitory effect of all four tea variety extracts against bacterial and fungal pathogens are indicated in Table 3. All three bacterial strains (*S. aureus*, *S. abony* and *E. coli*) are more sensitive than fungal strains (*A. flavous*, *A. brasiliensis* and *C. albicans*). Among bacterial strains *E. coli* was found to be more sensitive with large inhibition diameter in Darjileeng tea (12 mm) followed by Nilgiri (9.5 mm), Kangra (8.5 mm) and Assam (7 mm). Green tea extracts have poor but positive antimicrobial effect in all three fungal strains. Among all four tea varieties, only Darjileeng and Nilgiri tea extracts showed positive inhibitory effect among all fungal strains. Result of Almajano *et al.*[7] added important information about tea type (non-fermented, fermented and semi fermented) whose composition plays an important role in the study of antimicrobial activities. According to the findings, non-fermented tea (green tea) gives positive result against bacterial strains and the results of present study are in agreement with it.

In comparison to all the processed teas (Black, green and oolong), green tea has been found to be a rich source of total phenols, flavonoids with high antioxidant activity[20]. Result of present study gives confirmation regarding presence of higher phenol and flavonoid content responsible for high antioxidant activity. Yield of phenol content was found to be increased with increasing pressure up to 150 bar in PLE. Among all four variety of tea extracted, Darjileeng tea recorded to be highest for its oleoresin (23.48±0.53%) and phenol content (607.03±16.21 mg GAE $g^{-1}$). Widowati *et al.*[23] reported that green tea (22.11%) exhibits higher extraction yield of oleoresin as compared to black tea (20.14%) and oolong tea (17.39%). Instead of using methanol[23], present study was conducted with food grade ethanol as an extraction solvent in pressurized extraction medium. Shortened extraction time, reduced solvent consumption and improved extraction yield as compared to earlier used old extraction methods (soxhlet and maceration) enhances applicability of pressurized liquid extraction method in the area of extraction of target oriented active compounds from plant matrices.

Rusak *et al.*[21] compared phenolic content and antioxidant activity of white and green tea. According to their findings, solvent type used, extraction conditions and form of tea (bagged or loose) taken for extraction affected on the polyphenol and flavonoid content of tea. They observed that

green tea have more phenolic potential than white tea. They also confirms that addition of lemon juice in extraction solvent (water) could be the effective way to enhance extraction efficacy of phenolics and flavonoids in white tea. Use of 40% ethanol in extraction of catechins enhances the yield of EGCG effectively. In present study, catechin and EGCG were observed to be the dominant catechins in all four variety tea extracts which confirms the results reported by Rusak *et al.*[21] for EGCG.

Gadkari *et al.*[24] used supercritical $CO_2$ extraction method to extract polyphenols from fresh frozen tea leaves. All the experiments for polyphenol yield optimization were carried out at varying pressure (150-350 bar) and temperature (40-60°C) with using $CO_2$ and ethanol as an extraction solvent. Extraction time was kept constant (15 h) during all experiments. According to their findings, 250 bar pressure, 50°C temperature and 15 h extraction time was found to be the suitable optimum condition for maximum yield of polyphenols. EGCG was observed to be the predominant catechin among others with the marked increasing concentration level in the range of 700-850 mg $g^{-1}$. In present study, concentration of EGCG found to be lower (30.47 mg $g^{-1}$ for Darjileeng tea) as compared to the result reported by Gadkari *et al.*[24]. This lower concentration of EGCG in present study may be due to applied extraction parameters like short time (1 h), solvent (ethanol), pressure range (50-200 bar), tea variety and adopted extraction method (PLE).

In addition to supercritical $CO_2$ extraction method, Gadkari *et al.*[25] also adopted solid-liquid and liquid-liquid extraction method for extraction and evaluation of chemical composition of garden tea leaves. Different solvents were used for extraction catechins and caffeine compounds. Result of this study showed that 95% ethanol exhibits good solvent in the case of freeze dried quick mechanically expelled tea leaf juice (FD-QMETLJ) which gives total polyphenol content in the range of 350-450 g GAE $kg^{-1}$. These results for total polyphenol content (401.81-607.03 mg GAE $g^{-1}$) of green tea extracts (extracted by PLE with ethanol as extraction solvent) found to be comparable with the previously reported results for FD-QMETLJ. However, DPPH percent inhibition activity of present study for all four variety tea extracts were found to be more (59.63-74.40%) than the previously reported DPPH activity for FD-QMETLJ (16.97-20.83%).

Cui *et al.*[26] suggested a very environment friendly solvent, generally regarded as safe and alternative to organic solvent known as β-cyclodextrin (cyclic oligosaccharide) to extract phenolic compounds from tea leaves. In addition to aqueous β-Cyclodextrin (β-CD), two other solvents namely water and 50% ethanol were also used to compare yield of polyphenols with β-CD. The β-CD used in different concentrations (5-30 g $L^{-1}$), temperatures (20-70°C) and time (20-120 min) in order to optimize yield of polyphenols. Result of this study showed that yield of EGCG and ECG were observed to be higher (at concentration 15 g $L^{-1}$) as compared to water and 50% ethanol. Optimized level for β-CD was noted at a condition of 25 g $L^{-1}$concentration, 60°C temperature and 60 min extraction time where maximum yield of EGCG (118.7 mg $g^{-1}$) and ECG (54.6 mg $g^{-1}$) observed. In comparison to results of Cui *et al.*[26] for yield of EGCG and ECG, this study PLE method give poor yield for EGCG (24.75-30.47 mg $g^{-1}$) and ECG (4.24-8 mg $g^{-1}$). Moreover, used solvent ethanol also act as poor solvent in extracting polyphenols completely from green tea.

In a different study, Karagozlu *et al.*[27] investigated comparative effect of both black and green tea antioxidants in dairy product kefir after its value addition. Both phenol concentration and DPPH antioxidant activity of green tea extract supplemented kefir was observed to be more than black tea supplemented kefir. Effect of increasing concentration of green tea extract from 2-4% in kefir was also observed to be beneficial in terms of improving nutritional value of kefir.

Antimicrobial study of all four variety green tea extracts showed positive results in bacterial and fungal strains investigated in present study. As compared to fungal strains, strong inhibitory activity were observed in all bacterial strains in case of all the four varieties of tea extracts. Extracts of Darjileeng tea followed by Nilgiri tea gives more inhibitory activity while bacterial strains of *E. coli* followed by *S. aureus* were the affected most. In a different study Almajano *et al.*[7] studied antimicrobial effect of fermented tea (black tea), semi fermented tea (oolong and red tea), non-fermented tea (green and white tea) and tea infusions. Almajano *et al.*[7] prepared all tea samples by extracting it in boiling water with 5 min extraction time. Result of this study revealed that non-fermented tea have strong antimicrobial effect than other two type. According to their findings, investigated tea samples with higher polyphenol content and antioxidant activities exhibits highest antimicrobial activity. In present study Darjileeng tea with highest polyphenol concentration showed good antimicrobial activity in both bacterial and fungal strains. In the case of strain *E. Coli*, pressurized liquid extracts of all four variety green tea gives strong inhibitory activity (ranging 7-12 mm) than the previous observed results where average inhibition zone of *E. Coli* for green tea observed to be 6.2 mm[7].

Nibir *et al.*[28] comparatively assessed four tea varieties of Bangladesh (flowery broken orange pekoe, broken orange pekoe, red dust and green tea) for total polyphenol content, antioxidant activity and antimicrobial activity. All aqueous sample extracts were prepared by boiling tea powder in distilled water for 45 min. Results of this study indicate that green tea variety have the highest total polyphenols, flavonoids, antioxidant and antimicrobial activity among the other investigated tea varieties. In comparison to total phenol content, Bangladesh green tea variety gives lower value (26.33 mg GAE $g^{-1}$) than the value reported in present study while flavonoid content found to be more (50.12 mg catechin $g^{-1}$) in Bangladesh green tea variety. Difference between flavonoid content in both the studies may be due to the use of changing standards (Quercetin and Catechin) to measured flavonoid activity. Antimicrobial activity was measured against nine bacterial strains among which strains of *E. coli* and *S. aureus* matches with the present study. Due to higher concentration (100 mg $mL^{-1}$) of used green tea extracts for antimicrobial study, zones of inhibition for *E. coli* and *S. aureus* screened at a range of 14 and 19 mm, respectively.

Dubey and Mehta[29] extracted commercial Darjileeng green tea with the help of cold extraction method to study the antimicrobial activity. Agar well diffusion method and three bacterial strains (*S. aureus*, *E. coli* and *P. aeruginosa*) were used to determined antimicrobial activity. Inhibition zone diameter for all bacterial strains were measured at different aqueous green tea extract concentrations (12.5-200 mg $mL^{-1}$). At lowest concentration (12.5 mg $mL^{-1}$) inhibitory activity of *S. aureus*, *E. coli* and *P. aeruginosa* observed to be 8, 10 and 10 mm respectively. In present study, bacterial strain of *E. coli* inhibited more (7-12 mm) in all four variety green tea extracts at a concentration of 10 mg $mL^{-1}$. In comparison to *S. aureus* inhibitory activity of previous study, present study shows lower range of inhibition zone (5-7.5 mm at 10 mg $mL^{-1}$ concentration) in all four variety green tea extracts. Therefore, it can be concluded that extraction solvent, extraction time and extraction method play an important role on the antioxidant and antimicrobial activity of tea extracts.

## CONCLUSION

Pressurized liquid extraction technique applied in present study gives better yield of green tea extracts at optimized conditions of 150 bar pressure, 3 mL $min^{-1}$ flow rate and 75°C temperature of extraction solvent (ethanol). Extraction yield was optimized on the basis of measured total phenol content of extracts obtained at the end of each experiment. Result of Darjileeng tea variety was proved to be the best in terms of polyphenol yield, catechins concentration, DPPH antioxidant activity and antimicrobial study. Catechin and EGCG are the stronger catechins among the group of five catechins which was available in large concentration. Extracts of all four varieties give poor performance against fungal strains and strongly inhibit bacterial strains. Green tea has a very high antioxidant potential and can find wide applications in food and other nutraceutical industries.

## SIGNIFICANCE STATEMENTS

Present study provides useful information regarding the green tea antioxidant compounds and other phenolic profile investigated for four Indian green tea varieties extracted by using pressurized liquid extraction method. Moreover, results of phenolic profile of green tea extracts extracted by pressurized liquid extraction method were reported first time and new HPLC method was developed for phenolic acids determination which will be helpful for new researchers. Results of antioxidant and antimicrobial study of green tea extracts revealed its potential use for value addition in food, beverage and nutraceutical products to enhance shelf life and overall antioxidant potential of final food products.

## ACKNOWLEDGMENTS

First author would like to thank Council of Scientific and Industrial Research (CSIR), New Delhifor financial support through CSIR-RA Fellowship No. (09/086(1268)/2017-EMR-I) to carry out this work. The authors also thank Dr. P. Hariprasad for giving valuable efforts and guidelines to conduct experiments of antimicrobial assay in their laboratory.The help rendered by Er. S. K. Satpathy, Asst. Prof., CAEPHT, Central Agricultural University, Gangtok, India for language editing of the manuscript is duly acknowledge.

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