EFFECT OF TYPES OF EXTRACTIONS ON ANTIOXIDANT ACTIVITY OF VARIETIES OF BLACK TEAS FROM INDIA

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Traditional extraction method
Magnetic stirring
Sonicator
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Theaflavins
Thearubigins
Radical scavenging activity
Caffeine

ABSTRACT

Tea sample (silver cloud dust) was extracted with water by traditional extraction method (TEM) at variable material: solvent ratio (1:20, 1:30, 1:40, 1:50), temperature (50, 60, 70, 80, 90°C) and time (10, 20, 30, 40 mins). Extracts prepared were analysed for antioxidant activity (AA) and total polyphenol content (TPP). The extract of 1:50, 80°C, 30 mins showed higher yield, TPP and RSA compared to other extracts. Extraction (1:50, 80°C for 10,20,30,40 mins) was also carried by using magnetic stirring and sonicator. TEM showed higher AA and TPP. Therefore different samples of tea dust and leaf were extracted by TEM. All black tea leaf showed higher caffeine content than dust. Darjeeling leaf exhibited higher yield, RSA, TPP and caffeine. TEM showed higher AA and TPP. Therefore different samples of tea dust and leaf were extracted by TEM. All black tea leaf showed higher caffeine content than dust. Darjeeling leaf exhibited higher yield, RSA, TPP and caffeine. The extract of 1:50, 80°C, 30 mins showed higher yield, TPP and RSA compared to other extracts. Extraction (1:50, 80°C for 10,20,30,40 mins) was also carried by using magnetic stirring and sonicator. TEM showed higher AA and TPP. Therefore different samples of tea dust and leaf were extracted by TEM. All black tea leaf showed higher caffeine content than dust. Darjeeling leaf exhibited higher yield, RSA, TPP and caffeine. TEM showed higher AA and TPP. Therefore different samples of tea dust and leaf were extracted by TEM. All black tea leaf showed higher caffeine content than dust. Darjeeling leaf exhibited higher yield, RSA, TPP and caffeine. TF ranged between 0.1-0.2 % while TR content was 3.8-6% for varieties of tea extracts. Nilgiri dust extract showed higher colour. Darjeeling leaf showed highest caffeine content (12%).

Abbreviations: TPP - Total polyphenol content, RSA - Radical scavenging activity, RPA - Reducing power assay, TFs – Theaflavins, TRs - Thearubigins, HPLC - High performance liquid chromatography and DPPH - 1,1-diphenyl-2-picrylhydrazil

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1 Introduction

Black tea (*Camellia sinensis*) is a common beverage consumed by 73-78% of the world population. Black Tea is prepared by processing by fermentation and drying of tea leaves. Factors like species, season, age of leaf, climate, and horticultural practices greatly influence the variation in composition of tea (Lin et al., 1996). The three major forms of tea are green tea, semi-fermented oolong tea and fermented black tea which differ in production process which influences their chemical constituents and taste. Regular intake of tea is associated with an improved antioxidant status in vivo that may contribute to the lowering risk of certain types of cancer, coronary heart disease, atherosclerosis, stroke (Geleijnse et al., 1999; Hollman & Katan, 1999; Hong et al., 2001; Uesato et al., 2001; Wu et al., 2004) reduced mutagenicity (Gupta et al., 2001; Kuroda & Hara, 1999) and inflammation (Katiyar et al., 1999), protection against neurodegenerative diseases (Choi et al., 2004; Datla et al., 2004) and increasing insulin sensitivity (Wu et al., 2004).

There are many polyphenols in tea such as catechins, flavonols, flavanones, phenolic acids, glycosides and the aglycones of plant pigments (Pan et al., 2003). During fermentation of tea leaves, an enzymatic oxidation of tea polyphenols takes place, especially tea catechins takes place leading to formation of a series of multimeric coloured chemical compounds, such as theaflavins (TFs) and thearubigins (TRs), which are responsible for the characteristics of the black tea. TF content is an important chemical compound in determining black tea quality (Roberts & Smith, 1963). Tea polyphenols (TPs) are natural antioxidants (Tanizawa et al., 1984) and show stronger antioxidative activity and lower toxicity than butylated hydroxy anisole, butylated hydroxy toluene and DL-tocopherol. Investigations have indicated that black teas containing smaller amounts of polyphenols also shown antioxidant activities (Tanizawa et al., 1984). Attempts are made to increase the extraction of polyphenols and yield in black tea. The purpose of the present investigation is to systematically study the effect of material: solvent ratio, time and temperature on traditional method of extraction and to compare with extracts from sonicator and magnetic stirring methods. Different samples of black tea and dust from north and southern part of India were extracted and analysed for quality and antioxidant activity affected by above stated variables.

2 Materials and Methods:

Different samples of tea dust and leaf viz., Nilgiri leaf, Nilgiri dust, Assam leaf, Assam dust, Kelagur leaf, kelagur dust and Darjeeling leaf were procured from local market of Mysore, India.

2.1 Standards and chemicals

All chemicals and solvents used are analytical grade, Gallic acid, 1,1-diphenyl-1-picrylhydrazyl (DPPH*), Butylated hydroxy anisole (BHA), Folin-Ciocalteu (FC-reagent), were procured from Sigma chemical.co., (St. Louis, Mo USA). Solvents (methanol, ethyl acetate) and Disodium hydrogen phosphate-anhydrous, oxalic acid, dihydrogen phosphate and sodium carbonate were obtained from Merck, Mumbai, India.

2.2 Preparation of tea extracts

2.2.1 Traditional method: variable extraction

2.2.1.1 Material: solvent ratio

Tea sample (Nilgiri dust) was extracted with water by traditional method by heating at 80°C for 30 minutes with different material: solvent ratio (tea: water) viz., 1:20, 1:30, 1:40 and 1:50. Hot extract was filtered through whatmann no.1 filter paper and the extract was desolventized on rota-vapour at 60°C under reduced pressure (40 milli bars) and the product stored at 4°C.

2.2.1.2 Time

Tea sample (Nilgiri dust) was extracted with water by traditional method by heating at 80°C and 10, 20 and 30 minutes at 1:50 material: solvent ratio. Hot extract was filtered through whatmann no.1 filter paper and the extract was desolventized on rota vapour at 60°C under reduced pressure (40 milli bars) and the product stored at 4°C.

2.2.1.3 Temperature

Tea sample (Nilgiri dust) was extracted with water by traditional method by heating at different temperature viz., 50, 60, 70, 80 and 90°C for 30 minutes at 1:50 material: solvent ratio. Hot extract was filtered through whatmann no.1 filter paper and the extract was desolventized on rota vapour at 60°C under reduced pressure (40 milli bars) and the product stored at 4°C.

2.2.1.4 Extraction by using sonicator

Tea sample (Nilgiri dust) was extracted with water (1:50, 80°C for 10, 20, 30, 40 mins) using sonicator with heating.

2.2.2 Extraction by using magnetic stirrer

Tea sample (Nilgiri dust) was extracted with hot water for 10, 20, 30 and 40 mins at 80°C with 1:50 material: solvent ratio using magnetic stirrer with heater.

2.2.3 Extraction using variety of tea samples

Different samples of tea dust and leaf viz., silver cloud leaf, silver cloud dust, Assam leaf, Assam dust, Kelagur leaf,
kelagur dust and Darjeeling leaf were used for extraction by traditional method in glass, filtered and desolventized.

2.3 Determination of Polyphenols

Samples were analyzed for total polyphenol content according to the Folin-Ciocalteu method (Singleton & Rossi 1965). The concentration was calculated using gallic acid as a standard, and the results were expressed as gallic acid equivalents per 100gm of extract.

2.4 Free Radical Scavenging Activity

Free radical scavenging activity was measured by the 1,1-diphenyl-2-picolrylhydrazil (DPPH) method (Oktay et al., 2003). All analyses were run in triplicate and the values were averaged. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

Radical scavenging activity (%) = (Control OD – Sample OD/Control OD) × 100

2.5 Reducing power assay (RPA)

The reducing power was determined according to the method of Oyaizu (Oyaizu 1986). 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide was mixed with Various concentrations of black tea extracts (2.5 ml) and incubated at 50 °C for 20 min. 2.5 ml of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was taken and mixed with 5 ml deionised water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

2.6 Spectrophotometric estimation for theaflavins (TFs) and thearubigins (TRs)

Biochemical assessment of tea quality was done by estimation of TFs and TRs of black tea. In the present studies, a rapid procedure for estimating theaflavins and thearubigins of black tea was adopted with modification (Ullah, 1986). The absorbance was measured on a UV-visible spectrophotometer (Cintra -10, Australia).

2.7 Estimation of caffeine by HPLC method

Different samples of black tea of dust and leaf viz., silver cloud leaf, silver cloud dust, Assam leaf, Assam dust, Kelagur leaf, kelagur dust and Darjeeling were used for extraction by traditional method in glass. Caffeine content in extracts was estimated by the HPLC method (Naik & Nagalakshmi, 1997).

HPLC analysis of extracts was carried out using waters HPLC system consisted of waters binary HPLC 515 pump, manual injector 7725 and UV detector. The column used was C18 (250 x 4.6 mm i.d. particle size 3 µm). The mobile phase consisted of acetonitrile and water (20:80 v/v) at a flow rate of 1 mL/min at a wavelength of 276 nm. Extract (1mg/ml) dissolved in hot water; filtered through 0.45µ filter was subjected to the cleanup procedure as described by Naik & Nagalakshmi (1997).

2.8 Statistical analysis

All the analyses were carried out in triplicate and the results were provided as mean value with standard deviation. The obtained data were subjected to statistical analysis and the means compared by Duncan’s New Multiple Range test (p<0.05) are presented.

Table 1 Effect of material: solvent, time and temperature on yield, RSA (%) and TPP (%) of black tea (Nilgiri dust) extracted with water by traditional method.

<table>
<thead>
<tr>
<th>Material: Solvent</th>
<th>Time (Minutes)</th>
<th>Yield (%)</th>
<th>TPP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 ppm</td>
<td>15 ppm</td>
<td>20 ppm</td>
</tr>
<tr>
<td>1:20</td>
<td>46.6±0.3a</td>
<td>62.9±0.3a</td>
<td>80.1±0.2a</td>
</tr>
<tr>
<td>1:30</td>
<td>53.7±0.2b</td>
<td>72.3±0.1b</td>
<td>86.4±0.1b</td>
</tr>
<tr>
<td>1:40</td>
<td>60.7±0.1c</td>
<td>82.8±0.1c</td>
<td>90.7±0.1c</td>
</tr>
<tr>
<td>1:50</td>
<td>62.5±0.3d</td>
<td>85.9±0.2d</td>
<td>91.3±0.1d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Yield (%)</th>
<th>TPP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>26±0.4a</td>
<td>47.7±0.2a</td>
</tr>
<tr>
<td>20</td>
<td>31.2±0.1b</td>
<td>57.6±0.1b</td>
</tr>
<tr>
<td>30</td>
<td>62.5±0.3d</td>
<td>85.9±0.3d</td>
</tr>
<tr>
<td>40</td>
<td>46.6±0.2a</td>
<td>50.9±0.1e</td>
</tr>
<tr>
<td>50</td>
<td>34.8±0.2a</td>
<td>55.7±0.1a</td>
</tr>
<tr>
<td>60</td>
<td>49.4±0.1d</td>
<td>70.6±0.1c</td>
</tr>
<tr>
<td>70</td>
<td>44.3±0.2b</td>
<td>76.6±0.1d</td>
</tr>
<tr>
<td>80</td>
<td>62.5±0.3e</td>
<td>85.9±0.2e</td>
</tr>
<tr>
<td>90</td>
<td>46.0±0.1c</td>
<td>57.1±0.3b</td>
</tr>
</tbody>
</table>

Values are mean ± SD of triplicates; Values having different superscripts are significantly (p<0.05) different.
3 Results and Discussion

3.1 Traditional method of black tea extraction

3.1.1 Effect of material: solvent ratio on traditional extraction of black tea

Tea sample (silver cloud dust) was extracted using different material: solvent ratio (1:20, 1:30, 1:40, 1:50). It was observed from the results that extraction carried with 1:50 ratio showed higher yield, RSA and TPP (26%, 91.3% at 20 ppm, and 32.5%) as compared to other ratios and also as the solvent ratio was increased there was increase in yield, RSA and TPP as shown in table 1. Therefore 1:50 material: solvent ratio was further used for other experiments.

3.1.2 Effect of time on traditional extraction of black tea

Extraction was carried for different period of time variation of 10, 20, 30, and 40 min with 1:50 material: solvent ratio. It was observed from the results that extraction carried for 30 min showed higher RSA and TPP, yield (86.1% at 20 ppm, 23.8%, 24%) as compared to other as shown in table 2.

3.1.3 Effect of temperature on traditional extraction of black tea

Extraction was carried at varied temperature viz., 50, 60, 70, 80 and 90°C with 1:50 material: solvent ratio for 30 minutes. It was observed from results that the extraction carried at 80°C exhibited higher RSA and TPP (91.3% at 20 ppm, 32.5%) as compared to other extraction as shown in table 1.

3.2 Extraction using magnetic stirrer

Extraction was carried for different period of time variation of 10, 20, 30 and 40 min with standardized material: solvent ratio using magnetic stirrer at 80°C. Results showed that extraction carried for 20 min showed higher RSA and TPP, yield (86.1% at 20 ppm, 23.8%, 24%) as compared to other as shown in table 2.

3.3 Extraction using sonicator

Extraction using sonicator was carried at 60°C for 10, 20 and 30 minutes. Extraction carried at 60°C using sonicator for 30 minutes showed higher RSA, TPP and yield (80.6% at 20 ppm, 24.3%, 23%) compared to other as shown in table 3.

3.4 Extraction using variety of tea samples

Different samples of tea dust and leaf viz., silver cloud leaf, silver cloud dust, Assam leaf, Assam dust, Kelagur leaf, kelagur dust and Darjeeling were used for extraction by traditional method at 80°C temperature for 30 min, 1:50 material-solvent ratio, filtered and desolventized. Darjeeling leaf extract exhibited higher RSA, TPP and yield compared to other varieties as shown in figure 1.
3.5 Reducing power assay of extract from variety of tea

Different samples of tea dust and leaf viz., silver cloud leaf, silver cloud dust, Assam leaf, Assam dust, Kelagur leaf, kelagur dust and Darjeeling were used for extraction by traditional method, filtered and desolventized. Darjeeling leaf extract exhibited higher RPA (0.37 at 20ppm) compared to other varieties as shown in figure 2.

3.6 Determination of TF and TR of t black teas

As a result of enzymatic oxidation of catechins and their subsequent condensation two groups of pigments, the golden yellow theaflavins (TF) and the reddish-brown thearubigin are formed during black tea manufacture which is responsible for liquor characteristics of tea (Ullah, 1986). Liquor characters like colour, brightness, strength, briskness and quality of teas associated with TF. The body of the liquor is mainly due to TR. Thus TF, TR measurement would serve as an indicator for quality control of tea. The results showed that the Assam dust extract had higher TF:TR while kelagur leaf and nilgiri dust exhibited higher total colour and total brightness as shown in table 4. TF ranged between 0.1-0.2% while TR content was 3.8-6% for varieties of teas. Tea dust showed higher colour and brightness than leaf. Nilgiri dust showed higher colour and brightness while Darjeeling leaf showed least value as shown in table 4.
Table 4 TF, TR and Total colour (TC) of extracts of black teas extracted by traditional method.

<table>
<thead>
<tr>
<th>Black Tea</th>
<th>TF (%)</th>
<th>TR (%)</th>
<th>Total Colour(%)</th>
<th>Caffeine content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilgiri leaf</td>
<td>0.12±0.03a</td>
<td>5.5±1.1c</td>
<td>1.1±0.2b</td>
<td>3.0±0.07b</td>
</tr>
<tr>
<td>Nilgiri dust</td>
<td>0.11±0.04d</td>
<td>5.1±1.5c</td>
<td>1.7±0.3b</td>
<td>2.5±0.06e</td>
</tr>
<tr>
<td>Assam leaf</td>
<td>0.21±0.02b</td>
<td>3.8±1.4a</td>
<td>2.0±0.4e</td>
<td>9.1±0.02d</td>
</tr>
<tr>
<td>Assam dust</td>
<td>0.24±0.05b</td>
<td>6.0±1.7d</td>
<td>2.2±0.2c</td>
<td>8.2±0.08e</td>
</tr>
<tr>
<td>Kelagur leaf</td>
<td>0.18±0.03b</td>
<td>4.2±1.9b</td>
<td>1.2±0.6b</td>
<td>11.2±0.05f</td>
</tr>
<tr>
<td>Kelagur dust</td>
<td>0.17±0.03b</td>
<td>4.6±1.6b</td>
<td>4.5±0.3d</td>
<td>10.5±0.06e</td>
</tr>
<tr>
<td>Darjeeling leaf</td>
<td>0.11±0.02a</td>
<td>4.1±1.8b</td>
<td>0.8±0.1c</td>
<td>12.0±0.02g</td>
</tr>
</tbody>
</table>

Values are mean ± SD of triplicates; Values having different superscripts are significantly (p<0.05) different.

3.7 Estimation of caffeine content of different tea sample

Different samples of tea dust and leaf viz., silver cloud leaf, silver cloud dust, Assam leaf, Assam dust, Kelagur leaf, kelagur dust and Darjeeling leaf extract samples were estimated for caffeine content by HPLC method. It was found that Darjeeling leaf sample contain high percent of caffeine (12%) as shown in table 4.

Conclusions

Tea sample (silver cloud dust) was extracted with water by traditional method by heating at different material: solvent ratio (1:20, 1:30, 1:40, 1:50), temperature (50, 60, 70, 80, 90°C), time (10, 20, 30, 40 mins). Extracts prepared were analysed for antioxidant activity and total polyphenol content (TPP). The extract of 1:50, 80°C, 30 mins in glass showed higher yield (26%), RSA (91.3% at 20 ppm) and TPP (32.5%) compared to other extracts. Hot (1:50, 80°C for 10,20,30,40 mins) extraction of tea was also carried by using magnetic stirring and sonicator. Hot extract of 20 mins showed higher yield, RSA at 20 ppm and TPP (24%, 86.1%, 23.8%) compared to sonicator but less than traditional extraction. Therefore different samples of tea dust and leaf viz., silver cloud leaf, silver cloud dust, Assam leaf, Assam dust, Kelagur dust, kelagur leaf and Darjeeling were extracted by traditional method and analyzed. Darjeeling leaf extract exhibited higher yield, RSA, TPP and caffeine (85% at 10ppm,24%, 12%). TF ranged between 0.1-0.2% while TR content was 3.8-6% for varieties of teas. Tea dust showed higher colour and brightness than leaf. Nilgiri dust showed higher colour. Darjeeling leaf showed highest caffeine content (12%) followed by Kelagur, Assam and nilgiri leaf and dust.

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References


Katiyar SK, Matsui MS, Elmets CA, Mukhtar H (1999) Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and


