Phytosterol Composition in the Oil of Bi-clonal Seed Stocks of Tea (*Camellia sinensis* L.) in North East India

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Abstract

The phytosterols are naturally occurring, physiologically active substances found in foods of plant origin. In present study, the phytosterols profile of four bi-clonal tea seed stock oils viz., TS 462, TS 464, TS 491, and TS 506 released from Tocklai are presented. Phytosterols were evaluated using GC-FID. Among the phytosterols, 24-methylene cycloartan-3-ol was recorded as the most abundant sterol in tea seed oil, accounting for 31% of total phytosterols. Results showed that the majority of the sterol differences were significant (p 0.05). TS 506 has the highest 24- methylene cycloartan-3-ol (476.03.7 mg kg$^{-1}$) followed by TS 491 (475.97 mg kg$^{-1}$). β-sitosterol, cycloartenol, canophyllol, lanosterol and campesterol were also present with their contents of 371.03 ± 0.26, 291.38 ± 0.12, 120.44 ± 0.13, 89.12 ± 0.19 and 69.05 ± 0.24 mg kg$^{-1}$ respectively. The outcomes of the present study provided a foundational understanding of the phytosterols profiles of the four bi-clonal tea seed oils and highlighted their similarities and differences.

Keywords: Bi-clonal, β-sitosterol, Phytosterols, Tea Seed, Oils

Introduction

More than 250 different sterols and related compounds collectively referred to as phytosterols or plant sterols, have been documented in a variety of plants of land and marine origin (Salehi *et al*., 2021). Vegetable oils, nuts, seeds, and cereals are all prevalent sources of phytosterols, which are naturally found in plant cell membranes which consist of stigmasterol, campesterol, and -sitosterol (Witkowska *et al*., 2021). Phytosterols have a wide range of biological effects, including antioxidant (Van Rensburg *et al*., 2000), anti-diabetic (Zaklos-Szyda, 2015), anti-atherosclerotic (Moghadasian, 2000), antibacterial, and antifungal effects, anti-inflammatory (Lopez-Garcia *et al*., 2019a), antieruptive, antihemolytic effects (Alvarez-Sala *et al*., 2018) and anticancer properties Lopez-Garcia *et al*., 2019b). Research has demonstrated that phytosterols can lower blood total cholesterol by preventing the absorption of low-density lipoprotein cholesterol (Plat *et al*., 2019).

Bi-clonal tea seeds are widely used as planting material for the commercial production of tea (Sarmah *et al*., 2018). At the moment, there is a lot of interest in product diversification and value addition. However, there is currently no systemic information available on the phytosterols profile of the bi-clonal tea (*Camellia sinensis* L.) seed oils that the Tocklai Tea Research Association (TRA) has released. This is the first comprehensive analysis of the phytosterols from the bi-clonal seed stock oil of North-East India origin. The purpose of this study...
was to identify the types of phytosterols and their concentration in the oils derived from bi-clonal seed stocks that were released by the TRA in North-East India. This research is the first to provide insights into expanding the uses of tea seed.

Materials and methods

Experimental

Four bi-clonal seed stocks of *Camellia sinensis* L. seeds were collected from Tocklai Tea Research Institute experimental site (Figure 1), New Botanical Area (26.75 °N latitude and 94.22 °E longitude) TRA, Jorhat district, South Bank, Assam. The seed stocks released by TRA to the tea industry are presented in Table 1 and Figure 1.

![Fig. 1. Tea Bi-clonal seed stocks](image)

**Table 1. TRA released bi-clonal hybrid tea seed stocks.**

<table>
<thead>
<tr>
<th>Stock no.</th>
<th>Year of release</th>
<th>Parents / generative clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS 462</td>
<td>1980</td>
<td>(TV1 x 124/48/8)</td>
</tr>
<tr>
<td>TS 464</td>
<td>1984</td>
<td>(TV1 x 19/29/2)</td>
</tr>
<tr>
<td>TS 491</td>
<td>1989</td>
<td>(TV1 x S.3A/1)</td>
</tr>
<tr>
<td>TS 506</td>
<td>1994</td>
<td>(TV1 x 19/22/4)</td>
</tr>
</tbody>
</table>

Tea seed oil extraction

The oil was extracted from freshly harvested dried cotyledons of matured tea seed of four different bi-clonal tea seed stocks that were kept for commercial multiplication following the method described by Lee and Yen (2006). Briefly, tea seeds were first roasted at 120 °C for 20 minutes to obtain tea seed oil, and then they were pressed using a twin screw extruder. Tea seed oil (100 g) was extracted with 200 mL of each of the following: acetone, methanol, ethanol, ethyl acetic acid, and acetonitrile for an hour at room temperature in a shaking incubator. The extracts were filtered, and the leftover material was used for extraction three more times following the same protocol. The combined filtrates from each solvent were evaporated in a rotary evaporator under vacuum at a temperature of 40 °C, and then the mixture was weighed to determine the extraction yield before being kept at -20 °C until use.

Gas chromatographic analysis

Sterol determination in seed oil was determined by the method of Association of Official Analytical Chemists (AOAC) 994.10 (1996). Quantification of phytosterols was done using the gas chromatographic (GC) system with capillary column (30 m x 0.250 mm x 0.15 mm, Agilent Technologies Inc., 5301 Stevens Creek Blvd Santa Clara, CA95051 United States). Analyte mixture of one microliter was injected into GC system with a split/splitless injector and flame ionization detector (FID, Agilent Technologies Inc. 5301 Stevens Creek Blvd Santa Clara, CA95051 United States). The split ratio was 10:1, and the inlet temperature was 250 °C. Helium was the carrier gas, flowing at a constant rate of 2.5 mL min⁻¹. Initially, the oven was set to start at 250 °C for 5 minutes, and then increased by 5 °C min⁻¹ up to 260 °C for 8 minutes. The detector was adjusted to 300 °C with 200 mL min⁻¹ of airflow, 80 mL min⁻¹ of hydrogen flow, and 40 mL min⁻¹ of helium makeup flow. The entire GC analysis took 15 minutes. SPE Fractionation of Sterols was carried out by Azadmard-Damirchi and Dutta (2006).

Phytosterol estimation

A standard calibration curve was created using the internal standard peak area to analyte peak area ratio at each concentration level. Each analyte was given its own calibration curve for calculation.
purposes. High-level test solutions were diluted to fall within the permitted range. Measured as mg Phytosterols/100 g test portion, the amount of each phytosterols component in test portions was determined.

Methanol, ethanol, and other chemicals used were of HPLC grade and were purchased from Sisco Research Laboratories Pvt. Ltd. in Mumbai, India. All the standards for phytosterols profiling (standard mixture) were procured from Sigma-Aldrich (USA).

### Statistical Analysis

Values were calculated in triplicate for each analysis, and the mean ± SEM was used to express all results. With the use of the Statistical Package of WASP 2.0 (ICAR), a one-way analysis of variance and Duncan's test were used to determine whether a difference was statistically significant at a p-value of 0.05.

### Results and Discussion

In the current investigation, thirteen phytosterols in four bi-clonal tea seed stock oils were quantified (Table 1). According to Patel et al. (2020), the oil content of bi-clonal seed stocks ranged from 20.84 to 21.90%. Four bi-clonal tea seed stock oils varied in their phytosterols level and composition (Table 2). Thirteen compounds were identified in all of the examined bi-clonal seed stock oils, including b-amyrin, lupeol, campesterol, spinasterol, β-sitosterol, canophyllol, cycloartenol, stigmast-7-en-3-ol, betulin, lanosterol 24-methylene cycloartan-3-ol, lupanol and obtusifoliol. Three of the main ones were cycloartenol, -sitosterol, and 24-methylene cycloarten-3-ol. Further it was observed that the seed stock TS 506 oils had the highest lanosterol contents (90.44 mg kg⁻¹), and these compounds are effective in preventing cardiovascular disease (Lanzani et al., 2016) and delaying senescence (Lim et al., 2011). In addition, lanosterol is another significant non-polar active compound. However, it was found that several samples had unique sterol compositions. Both Camellia sinensis and Camellia oleifera seed oils had been found to have b-amyrin, β-sitosterol, and lanosterol (Li et al., 2022). All these compounds reported as above in the bi-clonal tea seed stock oil for the first time.

### Table 2. Phytosterols (mg kg⁻¹) of bi-clonal tea seed stock oils

<table>
<thead>
<tr>
<th>No</th>
<th>Phytosterols</th>
<th>TS 462</th>
<th>TS 464</th>
<th>TS 491</th>
<th>TS 506</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>b- Amyrin</td>
<td>30.17±0.39&quot;</td>
<td>30.46±0.15&quot;</td>
<td>31.55±0.25&quot;</td>
<td>31.14±0.23&quot;</td>
</tr>
<tr>
<td>2</td>
<td>Lupeol</td>
<td>9.45±0.82&quot;</td>
<td>8.40±0.04&quot;</td>
<td>8.69±0.10&quot;</td>
<td>8.59±0.18&quot;</td>
</tr>
<tr>
<td>3</td>
<td>Campesterol</td>
<td>67.17±1.14&quot;</td>
<td>68.28±1.00&quot;</td>
<td>68.97±0.37&quot;</td>
<td>69.05±0.24&quot;</td>
</tr>
<tr>
<td>4</td>
<td>Spinasterol</td>
<td>9.74±0.97&quot;</td>
<td>11.64±0.11&quot;</td>
<td>12.00±0.19&quot;</td>
<td>11.92±0.20&quot;</td>
</tr>
<tr>
<td>5</td>
<td>β-sitosterol</td>
<td>368.41±1.53&quot;</td>
<td>370.57±0.46&quot;</td>
<td>370.69±0.21&quot;</td>
<td>371.03±0.26&quot;</td>
</tr>
<tr>
<td>6</td>
<td>Canophyllol</td>
<td>120.00±0.38&quot;</td>
<td>120.04±0.36&quot;</td>
<td>120.77±0.25&quot;</td>
<td>120.44±0.13&quot;</td>
</tr>
<tr>
<td>7</td>
<td>Cycloartenol</td>
<td>286.08±4.29&quot;</td>
<td>289.51±1.17&quot;</td>
<td>291.04±0.58&quot;</td>
<td>291.38±0.12&quot;</td>
</tr>
<tr>
<td>8</td>
<td>Stigmas-7-en-3ol</td>
<td>31.68±0.45&quot;</td>
<td>31.78±0.03&quot;</td>
<td>31.99±0.27&quot;</td>
<td>31.94±0.30&quot;</td>
</tr>
<tr>
<td>9</td>
<td>Betulin</td>
<td>45.28±0.32&quot;</td>
<td>46.09±0.10&quot;</td>
<td>46.08±0.33&quot;</td>
<td>46.10±0.27&quot;</td>
</tr>
<tr>
<td>10</td>
<td>Lanosterol</td>
<td>89.44±0.76&quot;</td>
<td>89.40±0.05&quot;</td>
<td>89.89±0.39&quot;</td>
<td>89.12±0.19&quot;</td>
</tr>
<tr>
<td>11</td>
<td>24- Methylene cycloarten-3-ol</td>
<td>474.84±0.82&quot;</td>
<td>475.30±0.36&quot;</td>
<td>475.97±0.53&quot;</td>
<td>476.03±0.42&quot;</td>
</tr>
<tr>
<td>12</td>
<td>Lupanol</td>
<td>8.01±0.11&quot;</td>
<td>8.07±0.16&quot;</td>
<td>8.28±0.33&quot;</td>
<td>8.14±0.11&quot;</td>
</tr>
<tr>
<td>13</td>
<td>Obtusifoliol</td>
<td>3.68±1.18&quot;</td>
<td>3.67±0.06&quot;</td>
<td>4.09±0.20&quot;</td>
<td>3.93±0.36&quot;</td>
</tr>
<tr>
<td>Total sterols</td>
<td>1544.98</td>
<td>1553.23</td>
<td>1559.12</td>
<td>1558.82</td>
<td></td>
</tr>
</tbody>
</table>
The tea seed oil contains phytosterols in varying levels and compositions, similar to those found in vegetables, cereals, fruits, berries, nuts, and vegetable oils. Total Phytosterols in the bi-clonal tea seed oil tested were in the following order: TS491 > TS506 > TS464 > TS 462. Bi-clonal tea seed stocks oil has a higher total sterol content than vegetables, cereals, fruits, and berries, but it is lower than that of nuts and vegetable oils. Tea seed oil contains significant amounts of β-sitosterol and lanosterol, which can delay aging and prevent cardiovascular disease. These properties imply that tea seed oil could potentially be used as a nutraceutical and a raw material for cosmetics in the near future. This is the first report to study the phytosterols profile of tea seed oil obtained from bi-clonal tea seed stocks of North East India.

**Conclusion**

The tea seed oil contains phytosterols in varying levels and compositions, similar to those found in vegetables, cereals, fruits, berries, nuts, and vegetable oils. Total Phytosterols in the bi-clonal tea seed oil tested were in the following order: TS491 > TS506 > TS464 > TS 462. Bi-clonal tea seed stocks oil has a higher total sterol content than vegetables, cereals, fruits, and berries, but it is lower than that of nuts and vegetable oils. Tea seed oil contains significant amounts of β-sitosterol and lanosterol, which can delay aging and prevent cardiovascular disease. These properties imply that tea seed oil could potentially be used as a nutraceutical and a raw material for cosmetics in the near future. This is the first report to study the phytosterols profile of tea seed oil obtained from bi-clonal tea seed stocks of North East India.

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**Data Availability Statement:** Not applicable.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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Lopez Garcia, G. et al. 2019b. Effect of a Milk-Based Fruit Beverage Enriched with Plant Sterols and/or Galactooligosaccharides in a Murine Chronic