Short communication

Comparative in vitro antibacterial evaluation of different extracts of Camellia sinensis leaves form different geographical locations in India

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A B S T R A C T

Introduction: The present study was conducted to assess and compare the in vitro antibacterial property of four different solvent extracts from Camellia sinensis (tea) leaves collected form five different geographical regions of India.

Methods: All the extracts at the concentration of 4 mg/ml was tested for their in vitro antibacterial potential by cup plate method against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Staphylococcus epidermidis.

Results: All the test tea extracts were found to exhibit remarkable antibacterial effect except against S. aureus. The tea sample form Assam was found to be the most active.

Conclusion: The marked and differential antibacterial effect of C. sinensis leaf from different Indian locations was plausibly due to geographical variations in chemical constituents especially polyphenol contents of C. sinensis leaf.

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1. Introduction

The use of higher plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin.1 These investigations have been triggered by the emergence and spread of antibiotic resistant microorganisms causing the effective life-span of existing antibiotics limited.2 Hence, the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of newer anti-infective agents.3

Camellia sinensis L. (Theaceae), commonly known as tea is a large evergreen shrub indigenous to Eastern Asia where it is cultivated extensively. Tea is actually a product made from leaf and bud of the plant. C. sinensis, is the second most consumed beverage in the world.4 The dried cured leaves of C. sinensis have been used to prepare beverages for more than 4000 years. The method of curing determines the nature of the tea to be used for infusion. Green tea is a type of cured tea that is 'non fermented' and produced by drying and steaming the fresh leaves; whereas black tea leaves are withered, rolled, fermented and then dried.5 C. sinensis leaf has been used medicinally for centuries in the Traditional Chinese Medicine (TCM). Recently there has been renewed interest on green tea in prevention of several disease risks and other important health benefits.6 Previous researchers have reported several pharmacological and toxicological properties of C. sinensis leaf on animals and humans.7–10 The present study was conducted to assess and compare the in vitro antibacterial property of different extracts from tea leaves collected form different geographical regions of India.

2. Materials and methods

2.1. Plant material

The mature green leaves of C. sinensis L. (Theaceae) were collected during the month of August 2008 from five different locations of five states in India. The sources of the tea leaves were from the Darjeeling (West Bengal), Guwahati (Assam), Coonoor (Tamil Nadu), Coorg (Karnataka) and Munnar (Kerala) regions of India. Just after collection, the tea leaves were shade dried at room temperature (24–26 °C) and ground mechanically into a coarse powder.

2.2. Chemicals

All the chemicals used were of analytical grade, obtained from Merck. The culture media were obtained from Himedia.
The powdered leaves of green tea (40 g) were extracted separately with different solvents namely ethyl acetate, ethanol, methanol and aqueous methanol (60:40) by boiling under reflux for 8 h. All the extracts were concentrated by distilling off the solvents at reduced temperature using vacuum. Then the dry extracts were weighed and percentage of different extractive values was calculated with respect to the air dried powdered plant material. These results are presented in Table 1.

2.4. Test microorganisms

Standard bacterial cultures of Bacillus subtilis (NCIM-2708), Staphylococcus aureus (NCIM-2079), Escherichia coli (NCIM-2685) and Staphylococcus epidermidis (NCIM-2478) were obtained from Al-Ameen Biotechnology and Research center, Bangalore 560027, India. The microorganisms were maintained in usual laboratory conditions by sub-culturing at regular intervals.

2.5. Preparation of inoculums

A 24 h old culture was used for the preparation of bacterial suspension. The suspension of bacteria was made in sterile isotonic saline (0.9% w/v) solution. The turbidity of bacterial cultures was adjusted with sterile saline according to 0.5 McFarland turbidity standard (1.5 × 10⁶ cells/ml), for preparation of the inoculums.

2.6. Test samples

Test samples for in vitro antibacterial bioassay were prepared freshly from the dry extracts. All the five extracts obtained from different samples C. sinensis were dissolved in 10% w/w of dimethyl sulfoxide (DMSO) to get a concentration of 4 mg/ml. Ampicillin was used as reference and its solution was prepared at a concentration of 2 mg/ml in sterile distilled water.

2.7. Evaluation of antibacterial activity

The antibacterial activities of the test extracts were evaluated by cup plate method as reported by previous workers, with minor modifications. Nutrient agar medium previously prepared and sterilized was cooled down to approximately 45–50 °C. 20–25 ml of this media were poured into 9 cm sterile glass Petri dishes previously marked suitably at the bottom surface, to a depth of approximately 4 mm. The inoculum (1% of medium) was added to the molten agar media at 45 ± 0.5 °C in the Petri dishes and the plates were swirled gently to disperse the microorganisms homogeneously. The plates were then allowed to solidify. Then 4–5 bores were made on the medium by using sterile borer under aseptic conditions. 0.1 ml of the different extract samples was added to the respective bores. Similarly, 0.1 ml of ampicillin solution was employed as reference. 10% DMSO was used as control. The Petri dishes were kept in the refrigerator at 4 °C for 1 h for diffusion. After diffusion the Petri dishes were incubated at 37 ± 1 °C for 24 h and the zones of inhibition were observed and measured using a zone recorder in mm. Antibacterial activity of all the extracts was carried out against all four organisms in similar manner. All the experiments were performed in triplicate and results averaged.

3. Results and discussion

The antibacterial activity of four different solvent extracts from five C. sinensis leaf samples against four bacterial strains was assessed by cup plate method. This method is based on diffusion of antimicrobial component from reservoir hole to the surrounding inoculated nutrient agar medium, so that the growth of microorganisms is inhibited as circular zone around the hole. The results are presented in Tables 2–6. Based on the data obtained from the present study, except S. aureus all the three test bacteria were found to be sensitive against all the test tea extracts at the applied concentration of 4 mg/ml. No zone of inhibition was observed against S. aureus for all test extracts. However, the reference antibacterial agent ampicillin was effective against S. aureus at a concentration of 2 mg/ml. S. epidermidis was found to be the most sensitive against all test extracts whereas S. aureus was least sensitive (practically insensitive) against all the test tea extracts. The tea sample form Assam was found to be the most active. The semi polar solvent extracts namely ethanol and aqueous methanol (60:40) extracts of all five samples were found to be more effective in all cases. This indicated ethanol, water and methanol extracted maximum antibacterial principles (presumably polyphenols) form C. sinensis leaves. Different solvents have the capacity to extract different antimicrobial constituents from plants. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol, hydroalcohol or methanol extraction. Tea leaves contain varying amounts of polyphenols particularly flavonoids. Polyphenols are well known natural products known to possess several notable biological properties including excellent antimicrobial activity. The main flavonoids present in green tea leaves include catechins (flavan-3-ols) and most importantly epigallocatechin-3-gallate (EGCG). Tea leaf also contains some phenolic acids such as chlorogenic acid, gallic acid, caffeic acid, etc which also have antioxidant effect. The observed differences in antioxidant activity of tea leaf extracts could be attributed to the

Table 1

<table>
<thead>
<tr>
<th>Solvents</th>
<th>C. Assam</th>
<th>D. Darjeeling</th>
<th>T. Tamil Nadu</th>
<th>K. Karnataka</th>
<th>K. Kerala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>16.44</td>
<td>19.23</td>
<td>14.66</td>
<td>12.98</td>
<td>17.00</td>
</tr>
<tr>
<td>Methanol</td>
<td>22.09</td>
<td>34.12</td>
<td>24.86</td>
<td>25.94</td>
<td>26.52</td>
</tr>
<tr>
<td>Ethanol</td>
<td>30.41</td>
<td>49.37</td>
<td>25.22</td>
<td>28.37</td>
<td>30.99</td>
</tr>
<tr>
<td>Aqueous methanol</td>
<td>33.47</td>
<td>32.71</td>
<td>26.93</td>
<td>35.22</td>
<td>31.48</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition (mm)</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Aqueous methanol extract</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>NI</td>
<td>NI</td>
<td>17.9 ± 0.15</td>
<td>18.2 ± 0.42</td>
<td>22.2 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>10.03 ± 0.08</td>
<td>13.7 ± 0.26</td>
<td>14.8 ± 0.27</td>
<td>17.9 ± 0.13</td>
<td>21.03 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>13.7 ± 0.26</td>
<td>15.8 ± 0.31</td>
<td>14.8 ± 0.27</td>
<td>17.9 ± 0.13</td>
<td>21.03 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>12.2 ± 0.23</td>
<td>14.7 ± 0.23</td>
<td>16.5 ± 0.14</td>
<td>16.7 ± 0.26</td>
<td>25.4 ± 0.35</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as Mean ± Standard Error Mean (SEM); NI — No Inhibition.
Data are presented as Mean ± Standard Error Mean (SEM). NI = No Inhibition.

### Table 4
Antibacterial activity of *C. sinensis* extracts from Karnataka.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition (mm)</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Aqueous methanol extract</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>21.03 ± 0.10</td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>11.1 ± 0.05</td>
<td>12.7 ± 0.28</td>
<td>9.9 ± 0.17</td>
<td>10.16 ± 0.9</td>
<td>22.2 ± 0.23</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>12.91 ± 0.14</td>
<td>12.0 ± 0.08</td>
<td>14.2 ± 0.55</td>
<td>15.5 ± 0.17</td>
<td>25.4 ± 0.35</td>
<td></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>13.21 ± 0.17</td>
<td>14.1 ± 0.05</td>
<td>13.4 ± 0.14</td>
<td>14.1 ± 0.12</td>
<td>24.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as Mean ± Standard Error Mean (SEM). NI = No Inhibition.

### Table 5
Antibacterial activity of *C. sinensis* extracts from West Bengal (Darjeeling).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition (mm)</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Aqueous methanol extract</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>21.03 ± 0.10</td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>14.29 ± 0.06</td>
<td>14.1 ± 0.45</td>
<td>16.4 ± 0.65</td>
<td>17.4 ± 0.44</td>
<td>22.2 ± 0.23</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>12.91 ± 0.14</td>
<td>12.0 ± 0.08</td>
<td>14.2 ± 0.55</td>
<td>15.5 ± 0.17</td>
<td>25.4 ± 0.35</td>
<td></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>13.21 ± 0.17</td>
<td>14.1 ± 0.05</td>
<td>13.4 ± 0.14</td>
<td>14.1 ± 0.12</td>
<td>24.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as Mean ± Standard Error Mean (SEM). NI = No Inhibition.

### Table 6
Antibacterial activity of *C. sinensis* extracts from Kerala.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition (mm)</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Aqueous methanol extract</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>21.03 ± 0.10</td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>10.9 ± 0.23</td>
<td>11.4 ± 0.15</td>
<td>13.4 ± 0.15</td>
<td>14.3 ± 0.20</td>
<td>22.2 ± 0.23</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10.4 ± 0.08</td>
<td>13.3 ± 0.14</td>
<td>16.56 ± 0.14</td>
<td>16.6 ± 0.15</td>
<td>25.4 ± 0.35</td>
<td></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>11.21 ± 0.31</td>
<td>9.43 ± 0.08</td>
<td>15.43 ± 0.23</td>
<td>13.46 ± 0.12</td>
<td>24.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as Mean ± Standard Error Mean (SEM). NI = No Inhibition.

variations of composition in the polyphenol contents of *C. sinensis* leaves grown in different geographical locations due to diverse environmental or climatic factors.

The present preliminary study confirms remarkable in vitro antibacterial activity of four different solvent extracts of *C. sinensis* leaf collected from five different geographical locations against four bacterial strains. All the leaf extracts demonstrated marked antibacterial property against Bacillus subtilis, E. coli, and *S. epidermidis*. *C. sinensis* leaf collected from Assam was found to be the most active. The differential antibacterial effect of *C. sinensis* leaf from different locations of five Indian states was plausibly due to geographical variations in chemical constituents especially polyphenol contents of *C. sinensis* leaf.

### Conflicts of interest
All authors have none to declare.

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