PHYTOCHEMICAL SCREENING AND IN VITRO ANTIBACTERIAL ACTIVITY OF CAMELLIA SINENSIS

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ABSTRACT

The aim of the present work was to evaluate the phytochemical composition of Camellia sinensis and to assess the antibacterial activities of Camellia sinensis using in vitro antibacterial screening techniques. Extracts of leaves from the tea plant Camellia sinensis contain polyphenolic components with activity against a wide spectrum of microbes. Studies conducted over the last 20 years have shown that the green tea polyphenolic catechins, in particular (−)-epigallocatechingallate (EGCg) and (−)-epicatechingallate (ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency. The leaves were collected from the market and identified by the Pharmacognosy department of our own college. Phytochemical analysis revealed the presence of flavonoids. The study was carried out on various species of bacteria including E-coli (MTCC No.40), Staphylococcus aureus (MTCC No.87), Proteus vulgaris (MTCC No.742), Pseudomona saeruginos (MTCC No.424), Bacillus subtilis (MTCC No.441), Staphylococcus epidermidis (MTCC No.9041), and Micrococcus luteus (MTCC No.106), using cup plate method. The results obtained were compared against standard antibiotic streptomycin. The aqueous extract is effective against Proteus vulgaris and alcoholic extract is found effective against Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus. The ability of tea plant extract to inhibit the growth of bacterial strains is an indication of its antibacterial property that might be used in the management of bacterial infections in future.

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INTRODUCTION
In the world, phytomedicines have been used in past to treat various ailments long before the introduction of modern medicine. Herbal medicines are still widely used in many parts of the world especially in areas where people do not have contact to modern medicines [1, 2]. In most Asian countries where herbal medicines are still heavily relied upon because of high cost of chemotherapeutic drugs, there is a need for scientific research to estimate the biological activities of medicinal plants. The results obtained from such research may lead to the development and validation of traditionally used medicinally important plants and enable full usage of the properties of these plants [3]. Green tea is selected for the study because, tea consumption has its legendary origins in China of more than 4,000 years ago. Green tea has been used as both a beverage and a medicine in most of Asia, to help everything from controlling bleeding and helping heal wounds to regulating body temperature, blood sugar and promoting digestion[4].

The most abundant components in green tea are polyphenols, in particular flavonoids such as the catechins, catechingallates and proanthocyanidins [5]. Tea polyphenols are well-known for their antioxidant properties. Green tea has greater antioxidant potential than oolong and black teas [6-10]. Studies have shown that the strong antioxidant properties of green tea are attributed to catechins of EGCG and EGC[11-14] The three adjacent hydroxyl groups on the B-ring of EGCG, GCG, EGC, and GC are more effective in scavenging free radicals than the two adjacent OH groups of ECG, CG, and EC[15]. Black tea is also known to have potent antioxidant properties which are manifested by its ability to scavenge free radicals, inhibit lipid peroxidation, and chelate metal ions[16,17]. Although green tea has higher total phenolic content (TPC), free radical scavenging activity, and ferric reducing power, its ferrous ion-chelating ability is poorer than black tea.[9,10] Tea polyphenols are also known for their antibacterial activity. In general, antibacterial activity decreases when the extent of tea fermentation is increased, implying stronger activity in green tea than black tea.[18,19] Green tea catechins, particularly EGCG and ECG, have antibacterial activity against both Gram-positive and Gram-negative bacteria.[20-22] Green tea can prevent tooth decay by inhibiting oral bacteria [23]. The antibacterial activity of black tea has also been reported [18-20]. *Camellia sinensis* (*C. sinensis*), which is one of the most popular beverages worldwide, has been reported to have antimicrobial activities against various pathogenic bacteria[24-27], including MRSA[28] and MDR-*P. aeruginosa*[29,30]. There is no study on investigation of the antibacterial effects of *C. sinensis* against bacterial strains. Therefore, in this study, we investigated the antibacterial activity of the extract of green tea (*C. sinensis*) against bacterial strains.

MATERIAL AND METHODS
Collection of plant materials
The experiment was conducted in the year 2013 in the college. Leaves of *Camellia Sinesis* were collected from the Herbal Garden. The species was identified and authenticated at the Herbal department of Pioneer Pharmacy Degree College, Vadodara,Gujarat. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried for 3 days.

Source of microorganisms
The organisms studied, *Excherichia Coli* (MTCC No.40), *Pseudomonas aeroginosa*(MTCC No.424), *Staphylococcus aureus*(MTCC No.87), *Proteus vulgaris*(MTCC No.742), *Streptococcus mutans*(MTCC No.497), *Bacillus subtilus*(MTCC No.441), *Staphylococcus epidermidis*(MTCC No.9041), *Mirococcus luteus*(MTCC No.106). The organisms were obtained from the MTCC, Chandigarh. The Sub culturing was done after interval of 15 days.

Preparation of methanolic and aqueous leaf extract
Fresh leaves (500gm) of *Camellia sinensis* were shade dried at room temperature (32 – 35 °C) to constant weight over a period of 3 days. 45 g of the powdered leaves were separately extracted in 500 ml conical flasks with 90% methanol (methanolic extraction) and slightly warm water (aqueous extraction) for overnight. The extracts were separately filtered using sterile Whatman no. 1 filter paper. These extracts were used in further process.

Storage conditions
The extract was stored in a cool condition protected from direct sunlight.

Phytochemical analysis
Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was performed by the extracts.

Alkaloids [31]
Wagner’s test:- 1 ml of extract, add 2 ml of Wagner’s reagent (iodine in potassium iodide).
Dragendoff test:- 1 ml of extract, add 1 ml of Dragendorf’s reagent (potassium bismuth iodide solution).
Hager test:- 1 ml of extract, add 3 ml of Hager’s reagent (saturated aqueous solution of picric acid).
Mayer’s test:- 1 ml of extract, add 1 ml of Mayer’s reagent (potassium mercuric iodide solution).

Flavonoids:- 3 ml of each extract was added to 10 ml of distilled water the solution was shaken. 1 ml of 10% NaOH solution was added to the mixture. [32]

Saponins: 3 ml of each extract and dilute with 2 ml of distilled water was added in a test tube. The mixture was shaken vigorously.[33]
Salkowski Test:- 5 drops of concentrated H2SO4 were added to 1 ml of each extract in a separate test tube were boiled gently for 2 min and allowed to cool. 3 drop of ferric chloride solution were added to each extract. Glycosides-25 ml of dilute sulphuric acid was added to 5 ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5 ml of Fehling solution added.[32]
Reducing Sugars: To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling’s solution was added and heated over water bath. [32]

Terpenoids: Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly. [34]

**Determination of Antibacterial Activity**

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Briefly, Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells were made in the agar using the stainless steel borer of 6mm and filled with 200µl of plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 24 hours and the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition. Same method was applied for the standard antibiotic Streptomycin.

**RESULT AND DISCUSSION**

**Qualitative phytochemical analysis**

The present study reveals that plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthraquinones in different solvent extracts as shown in Table 1.

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Phytoconstituent</th>
<th>Aqueous extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Wagner’s test</td>
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<td></td>
<td>Mayer’s test</td>
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<tr>
<td></td>
<td>Hager’s test</td>
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<tr>
<td>2.</td>
<td>Carbohydrates</td>
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<tr>
<td></td>
<td>Molisch Test</td>
<td>-</td>
<td>-</td>
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<td></td>
<td>Benedicts Test</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3.</td>
<td>Glycosides</td>
<td></td>
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<tr>
<td></td>
<td>Legal test</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Baljet test</td>
<td>-</td>
<td>-</td>
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<tr>
<td>4.</td>
<td>Steroids</td>
<td></td>
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<tr>
<td></td>
<td>Lieberman Burchard Test</td>
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<td>-</td>
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<tr>
<td>5.</td>
<td>Proteins &amp; Amino acids</td>
<td></td>
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<tr>
<td></td>
<td>Biuret test</td>
<td>-</td>
<td>-</td>
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<td></td>
<td>Xanthoproteic test</td>
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<td>-</td>
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<td></td>
<td>Lead Acetate test</td>
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<td>-</td>
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<tr>
<td>8.</td>
<td>Saponins</td>
<td></td>
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<tr>
<td></td>
<td>Foam test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Flavonoids</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Antibacterial Activity**

Antibacterial activity of *Camellia sinensis* was seen against organisms namely *Escherichia coli* (MTCC No.40), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Pseudomonas aeruginosa* (MTCC No.424), *Bacillus subtilis* (MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041), and *Micrococcus luteus* (MTCC No.106). The methanolic extract exhibit effective antibacterial activity against the organisms like *Staphylococcus aureus*(2mm), *Bacillus subtilis*(3mm), *Micrococcus luteus*(2mm), *Staphylococcus epidermidis*(4mm) as shown in Table 2 while the Aqueous extract exhibits inhibition zone on *Proteus vulgaris* (4mm).Same procedure was also applied for the standard drug streptomycin and the results obtained are also presented in Table 2.

Table 1: Qualitative Phytochemical analysis of *Camellia sinensis*
Table 2 Antibacterial activity of Camellia sinensis and standard (Streptomycin)

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Standard (Streptomycin)</th>
<th>Diameter of zone of inhibition</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aqueous extract</td>
</tr>
<tr>
<td>E.coli</td>
<td>6 mm</td>
<td>-</td>
</tr>
<tr>
<td>S.aureus</td>
<td>6 mm</td>
<td>-</td>
</tr>
<tr>
<td>P.vulgaris</td>
<td>7 mm</td>
<td>4 mm</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>6 mm</td>
<td>-</td>
</tr>
<tr>
<td>B.subtilis</td>
<td>7 mm</td>
<td>-</td>
</tr>
<tr>
<td>S.epidermidis</td>
<td>6 mm</td>
<td>-</td>
</tr>
<tr>
<td>M.luteus</td>
<td>7 mm</td>
<td>-</td>
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</tbody>
</table>

CONCLUSION

Extracts of leaves from the tea plant Camellia sinensis contain polyphenolic components with activity against a wide spectrum of microbes. Studies conducted over the last 20 years have shown that the green tea polyphenolic catechins, in particular (−)-epigallocatechin gallate (EGCg) and (−)-epicatechin gallate (ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency. The study was carried out on various species of bacteria E.coli(MTCC No.40), Staphylococcus aureus (MTCC No.87), Proteus vulgaris (MTCC No.742), Pseudomonas aeruginosa (MTCC No.424), Bacillus subtilis (MTCC No.441), Staphylococcus epidermidis (MTCC No.9041), and Micrococcus luteus (MTCC No.106), using cup plate method. The results obtained were compared against standard antibiotic streptomycin. The zone of inhibition is seen in proteus vulgaris in aqueous extract and from alcoholic extract zone of inhibition was found in Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus. by getting the above results, author suggest further detail phytochemical investigation and work.

ACKNOWLEDGEMENT

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Authors’ Statements

Competing Interests

The authors declare no conflict of interest.

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