PHYSICAL SCREENING AND IN VITRO ANTIFUNGAL ACTIVITY OF CAMELLIA SINENSIS

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ABSTRACT

Objectives: The aim of the present work was to evaluate the phytochemical composition of Camellia sinensis and to assess the antifungal activities of Camellia sinensis using in vitro antifungal screening techniques. The ability of tea plant extract to inhibit the growth of fungal strains is an indication of its antifungal property that might be used in the management of fungal infections in future.

Methods: 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 polyphenol catechins, in particular (-)-epigallocatechingallate (EGCG) and (-)-epicatechingallate (ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative fungal 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 fungi including Candida albicans (MTCC No.183), Candida tropicalis (MTCC No.184) and Saccharomyces cerevisiae (MTCC No.170) using cup plate method. The results obtained were compared against standard antibiotic streptomycin.

Results: The aqueous extract is effective against Saccharomyces cerevisiae and very less against Candida albicans and no effect was found in Candida tropicalis. The alcoholic extract is found effective against Candida albicans and Saccharomyces cerevisiae and very less effect was seen against Candida tropicalis. Along with that it has a good source of Flavonoids.

Conclusion: The study illustrates that Camellia sinensis has shown a significant antifungal activity against various species of fungi like Saccharomyces cerevisiae, Candida albicans and very less antifungal effect was seen against Candida tropicalis; both in aqueous as well as methanolic extract.

Keywords: Green tea, Camellia sinensis Antifungal, Phytochemical screening, Zone of inhibition.

INTRODUCTION

In the world, phyto medicines 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 access 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, EG, and GC are more effective in scavenging free radicals than the two adjacent OH groups of EGC, 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 antifungal activity. In general, antifungal activity decreases when the extent of tea fermentation is increased, implying stronger activity in green tea than black tea [18, 19].

MATERIALS AND METHOD

Collection of plant materials
The experiment was conducted in the year 2013 in the college. Leaves of Camellia sinensis 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 Candida albicans (MTCC No.183), Candida tropicalis (MTCC No.184), and Saccharomyces cerevisiae (MTCC No.170). 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).

Dragendorff's test: 1 ml of extract, add 1 ml of Dragendorff'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 0.5 ml of plant extracts, 1 ml 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 Antifungal Activity

The antifungal 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 fungal inoculums on the media. Wells were made in the agar using the stainless steel borer of 8 mm and filled with 300 µl of plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 28°C for 72 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.

Antifungal Activity

Antifungal 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 Aqueous extract exhibits inhibition zone on *Proteus vulgaris* (4 mm). Same procedure was also applied for the standard drug streptomycin and the results obtained are also presented in Fig. 1. While the methanolic extract exhibit effective antifungal activity against the organisms like *Staphylococcus aureus* (2 mm), *Bacillus subtilis* (3 mm), *Micrococcus luteus* (2 mm), *Staphylococcus epidermidis* (4 mm) as shown in Fig. 2.

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 (−)-epigallocatechingallate (EGCg) and (−)-epicatechingallate (ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative fungal species with moderate potency. The study was carried out on various species of fungal *Candida albicans* (MTCC No.183), *Candida tropicalis* (MTCC No.184), and *Saccharomyces cerevisiae* (MTCC No.170) using cup plate method.

The results obtained were compared against standard antibiotic streptomycin. The results obtained were compared against standard antibiotic streptomycin. The zone of inhibition is seen in *Saccharomyces cerevisiae* and very less inhibition is seen against *Candida albicans* and no effect is found in *Candida tropicalis* inaqueous extract. The alcoholic extract is found effective against *Candida albicans* and *Saccharomyces cerevisiae* and very less effect was seen against *Candida tropicalis* by getting the above results, author suggest further detail phytochemical investigation and work.

Table 1: Qualitative Phytochemical analysis of *Camellia sinensis*

<table>
<thead>
<tr>
<th>S. 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>
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</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>-</td>
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<tr>
<td></td>
<td>Wagner’s test</td>
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<tr>
<td></td>
<td>Mayer’s test</td>
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<tr>
<td></td>
<td>Hager’s test</td>
<td>-</td>
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<td>2.</td>
<td>Carbohydrates</td>
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<td></td>
<td>Molisch Test</td>
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<tr>
<td></td>
<td>Benedicts Test</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3.</td>
<td>Glycosides</td>
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<td></td>
<td>Legal test</td>
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<td>-</td>
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<td></td>
<td>Baljet test</td>
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<tr>
<td>4.</td>
<td>Steroids</td>
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<td></td>
<td>Lieberman Burchard Test</td>
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<tr>
<td>5.</td>
<td>Proteins &amp; Amino acids</td>
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<tr>
<td></td>
<td>Biuret test</td>
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<td>-</td>
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<td></td>
<td>Xanthoproteic test</td>
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<td></td>
<td>Lead Acetate test</td>
<td>-</td>
<td>-</td>
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<tr>
<td>8.</td>
<td>Saponins</td>
<td></td>
<td></td>
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<td></td>
<td>Foam test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Flavonoids</td>
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<td></td>
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<tr>
<td></td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>
Fig. 1: Zone of Inhibition seen on various fungal species in aqueous extract of *Camellia sinensis*.

Fig. 2: Zone of Inhibition seen on various fungal species in methanolic extract of *Camellia sinensis*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


