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CAMELLIA SINENSIS LEAVES A NEW TREATMENT AGAINST URINARY TRACT INFECTION CAUSED BY *PSEUDOMONAS FLUORESCENS* AND *SERRATIA* SP

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ABSTRACT: Urinary tract infection is the most common disease in females and males which is big threat of kidney failure. The increasing interest is in the powerful biological activity of medicinal plant containing bioactive compounds which paves way for the importance to determine their antibacterial activity. The bioactive chemical determination revealed the presence of bioactive constituents' steroids, alkaloids, tannins and flavonoids due the color change in the reaction tubes. While the absence of terpenoids saponins and glycosides as there was no color change in the reaction tubes. The total flavonoid content was 16mg/gram while total phenolic compound was 0.9grams in the leave extract of *Camellia sinensis*. The reducing power was found 0.13grams/gram of leave extracts. The phenolic extract of *Camellia sinensis* showed the antibacterial activity against *Pseudomonas fluorescens* and *Serratia* Sp by showing maximum zone of inhibition around the bacterial colonies when compared with standard antibiotics Cotrimaxazole, Norfloxacin, Chloramphenicol, and Nalidixic acid.

INTRODUCTION: Medicinal plants are important source of pharmacological effects that act as new anti-infectious agents [1]. Plant leaves are the most commonly used traditionally natural antimicrobial agents against various drug resistant microorganisms for thousands of years by many cultures of peoples in the past decade [2]. Plant materials have shown the antimicrobial activity on various pathogenic microorganisms therefore consumption of tea has decrease the urinary tract infection [3]. The most important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides which have served a valuable starting material for drug development [4].

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Camellia sinensis a green tea is consumed worldwide and is second only to water in its popularity as a beverage and has ascribed many health benefits. It is generally safe, non-toxic having no side effects after use [6].

Serratia marcescens and *Pseudomonas aeruginosa* are pathogens associated with pyogenic infection and urinary tract infection [7]. *Camellia sinensis* possess antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids [8].

MATERIALS AND METHODS:

Collection of the sample: The fresh leaves of C*amellia sinensis* were collected [9] from the dense tea state garden at Ooty, Coimbatore district, Tamil Nadu South India.

Preparation of the extract: The leaves of fresh samples were cleaned and washed under running tap water [10]. The samples were dried in the oven at 37°C for 6 days.

After drying the samples were weighed and blended with warring blender and soaked with methanol [in ratio methanol: plant (6:1)] for 2 days and filtered using Whatman No. 1 paper. The methanol was completely removed by vacuum evaporator at 50°C till it gave a viscous mass. The crude extracts were weighed and stored at 4°C before analysis.

Preliminary Phytochemical Screening: The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar, tannins [11].

1. **Alkaloid test:** The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of 5% Sodium Hydroxide solution. The samples were then observed for the presence of turbidity or yellow color.

2. **Gallic tannin test:** 0.5 ml of extract was dissolved in 1 ml of water, mixed uniformly and then 2 drops of ferric chloride solution were added. The formation of blue color was observed for presence of gallic tannin.

3. **Catecholic tannin test:** 0.5 ml of extract was dissolved in 1 ml of water, mixed uniformly then 2 drops of ferric chloride solution were added and green black colour was observed for presence of catecholic tannin.

4. **Terpenoid test:** 4 mg 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 and red violet color was observed for the presence of terpenoids.

5. **Steroid test:** 4mg 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 and green bluish color was observed for presence of steroids.

6. **Glycoside test:** 0.5 grams of extract was dissolved in 2ml of glacial acetic acid and mixed well. To this few drops of ferric chloride and concentrated sulphuric acid were added then observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

7. **Flavonoid test:** 2ml of extract solution was treated with 1ml of lead acetate solution and white colour was observed for the presence of flavonoids.

8. **Saponins test:** 0.5ml of extract was treated with 5ml of distilled water and frothing persistence indicate the presence of saponins.

Determination of Total Flavonoids Content: The flavonoid content was estimated by [12] in which 0.5 ml of the sample was added to a test tube containing 1.5 ml of methanol. Then added 0.3 ml of 5% sodium nitrite solution and allowed to stand for 5 min. Added with 0.3 ml of 10% aluminium chloride, after 6 min 1 ml of 1 M sodium hydroxide was added and the mixture was diluted with distilled water.

The absorbance of the mixture at 510 nm was measured immediately. Apigenin was used as standard the flavonoid content was expressed as milligram catechin equivalents /g sample.

Determination of Total Phenolic Content: Determination of total phenolic content was carried out by taking [12] one hundred micro liters in 1ml of water methanol extract was dissolved in 1ml (1:1 ratio) of the Folin-Ciocalteu reagent. The solutions were mixed and incubated at room temperature for 1 minute. After 1 minute, 1ml of 35% sodium carbonate (Na_2CO_3) solution was added and makeup to 8 ml with distilled water.

The final mixture was shaken and then incubated for 1½ hour in the dark at room temperature. The absorbance of all samples was measured at 725 nm using colorimeter. Gallic acid was used as standard for the calibration curve and was plotted at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml gallic acid that was prepared in 80% (v/v) methanol. The absorbance was recorded at 725 nm using 80% (v/v) methanol as blank. Triplicate measurements were carried out and total phenolic content was expressed as milligram of gallic acid equivalents (GAE) per 100 gram of samples.

Assay of Reducing Power: The reductive capability of the extract was quantified by taking [12] one ml of methanolic extract was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. Similar concentrations of standard rutin were used as standard. The mixture was incubated at 50°C for 20 min. Then, the reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid.

Then, this was centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$. Blank reagent is prepared as above without adding extract. The absorbance was measured at 700 nm in a colorimeter against a blank sample. Ascarbic acid was used as standard the increased absorbance of the reaction mixture indicated greater reducing power.

Antimicrobial activity of *Camellia sinensis* leaves:

1. **Preparation of Inoculum:** The inoculum was prepared by [7] culturing the microorganisms in nutrient broth at 37°C for 12 hours to a concentration of approximately 105 CFU/ml was used for the antimicrobial analysis.

2. **Preparation of Extracts:** The dried powder of leaves was prepared by [10] taking 5g of leaves extracts and dissolved in 25ml of methanol and incubated for 24 hours at room temperature on constant shaking. After incubation the solution was filtered twice through Whatman no. 1 filter paper. The filtrate was evaporated to dryness at 50°C and then crude extracts were used for analyzing the antimicrobial activity of *Camellia sinensis* leaves.

3. **Agar-well Diffusion method:** The agar-well diffusion assay was adopted [10] for the present assay. Each bacterial suspension was spread over the surface of Mueller-Hinton agar (Himedia, India) plates containing 4 wells having 6 mm diameter. The wells were filled with 30µl each of the various concentrations (100µg, 200µg and 300µg) of extracts. The plates were incubated at 37°C for overnight. The results were expressed in terms of the diameter of the inhibition zone and Methanol used as control.

RESULT:

Identification of leaves: The leaves belongs to the Kingdom-Plantae, Order-Ericales, Family-Theaceae, Genus-*Camellia*, Species-*sinensis*.

Qualitative analysis of Phytochemicals: The extract showed the presence of phytochemicals namely alkaloids, gallic tannins, flavonoids, steroids and catecholic tannin by changing the color of the solution to yellow, white, green bluish, blue, green black respectively but indicated the absence of glycosides, saponins and terpenoid as there was no color change in the solution with respect to them.

Total Phytochemical contents: The total content of phenol was 0.9grams/gram of leave extracts while the total flavonoid content was 16mg/gram of leave extracts of *Camellia sinensis*. The reducing power was found 0.13grams/gram of leave extracts

Antimicrobial activity: The *Camellia sinensis* leaf extracts of various concentrations (100µg/ml, 200µg/ml and 300µg/ml) concentrations showed the zone of inhibition against gram negative bacteria *Pseudomonas fluorescens* (**Figure 1**) and *Serratia* Sp (**Figure 2**).

The zones of inhibition were measured in millimeter (mm) and then compared with antibiotic standard Cotrimaxazole, Norfloxacin, Chloramphenicol, and Nalidixic acid. The zone of inhibition was highest than them and susceptibility increased when the concentration was increased.

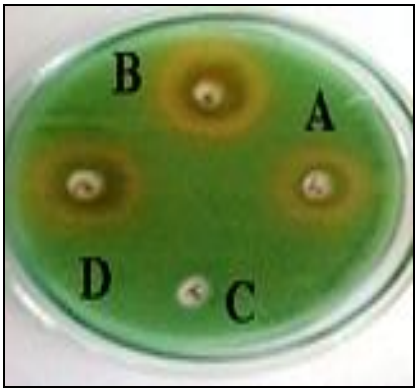

FIGURE 1: ANTIBACTERIAL ACTIVITY OF *CAMELLIA SINENSIS* LEAF EXTRACTS AGAINST GRAM NEGATIVE BACTERIA *PSEUDOMONAS FLUORESCENS* WHERE, C- CONTROL A- 100µg/ml, B- 200µg/ml and D- 300µg/ml). The zones of inhibition around colonies were measured in millimeter (mm).

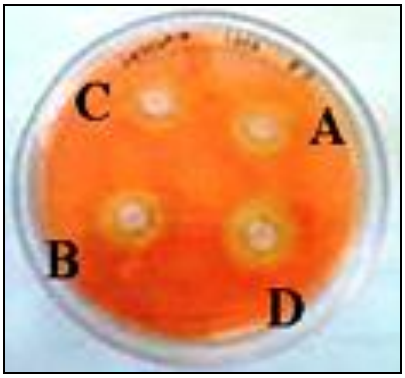

FIGURE 1: ANTIBACTERIAL ACTIVITY OF *CAMELLIA SINENSIS* LEAF EXTRACTS AGAINST *SERRATIA* Sp WHERE, C- CONTROL A- 100µg/ml, B- 200µg/ml and D- 300µg/ml). The zones of inhibition around colonies were measured in millimeter (mm).

DISCUSSION: The highest antimicrobial activity of tea is due to presence of catechins and polyphones which damages bacterial cell membrane [14]. They also serve in plant defense mechanisms to counteract reactive oxygen species in order to survive and prevent molecular damage and caused by microorganisms, insects, and herbivores [15]. The of bioactive compounds such as alkaloids, flavonoids, steroids, gallic tannins, catecholic tannin plays the vital role in the plant defense mechanisms [4, 8].

In this work, methanolic extract of *Camellia sinensis* had greater antibacterial activity against *Pseudomonas fluorescens* and *Serratia* Sp [16]. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria [17]. The active substance found in tea is supposed to reduce growth and development of microorganisms [18]. The antibacterial activity of *Camellia sinensis* leaf against *Listeria monocytogenes* by disc diffusion method, the methanolic extract had greater antibacterial property as compared to the water extract [9].

In the Gram negative outer membrane acting as a barrier to many environmental substances including antibiotics [19]. Spices in the past decade confirmed that the growth of both Gram-positive and Gram-negative food borne bacteria, yeast and molds can be inhibited [20]. Green tea leaf extracts tested in current study have also shown varying activities against environmental bacteria [3]. The green sorts of tea have shown higher antimicrobial activity than the black ones [21]. The antibacterial activity of *Camellia sinensis* tea extracts was selective and depends upon the concentration, type of the extracts and bacterial species [2].

In our work, different concentration of leaf extracts were used against two different pathogenic bacteria and highest zone of inhibition was observed against *Pseudomonas fluorescens* and *Serratia* Sp [5, 22].

CONCLUSION: *Camellia sinensis* leaves are having the duel benefits as medicinal values and food value. In this study we found that leaf extracts were found to be potential antibacterial agents against urinary tract and pyogenic infectious gram negative bacteria *Pseudomonas fluorescens* and *Serratia* Sp had shown the maximum zone of inhibition when compared with standard antibiotics Cotrimaxazole, Norfloxacin, Chloramphenicol, and Nalidixic acid.

Thus, *Camellia sinensis* leaves can be used an alternative medicine against the bacterial infection.

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