**PHYTOCHEMICAL PROFILING** **OF *CAMELLIA SINENSIS* METHANOLIC EXTRACT AND ITS ANTIBACTERIAL ACTIVITY**

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**ABSTRACT**

In the present study, phytochemical profiling of methanolic extract of *Camellia sinensis* was carried out using liquid chromatography coupled with a UV spectrophotometer (LC-UV) analysis. Besides, its antimicrobial potential has been evaluated against Gram-positive and Gram-negative two bacteria (*Bacillus subtilis* and *Escherichia coli* respectively). LC-UV analysis revealed the presence of four major compounds namely: Caffeic acid, Epicatechin, Syringic acid, and Epigallocatechin gallate. The extract showed a powerful antibacterial activity at the concentration of 5000 µg/ml with an inhibitory zone of 25.38±2.75 mm against *B. subtilis* ATCC 11778, and an inhibitory zone of 22.78±1.22 against *E. coli* ATCC 25922*.* This analysis revealed that *C. sinensis* leaves methanolic extract has strong antibacterial activity due to its high phenolic component content, and it may be considered a promising option for natural plant sources of antibacterial with high value.

**Keywords:** Antimicrobial; *Bacillus subtilis* ATCC 11778*; Camellia sinensis; Escherichia coli* ATCC 25922*;* LC-UV; Phytochemical.

**INTRODUCTION**

Bacillus species are Gram-positive or Gram-negative aerobic rod-shaped bacteria. They produce spores that are resistant to cold, heat, and standard disinfectants, allowing the bacteria to live in a variety of environments ([Cote et al. 2015](#_ENREF_3)). Bacillus is a large genus that contains over 200 species. The vast majority of Bacillus species are non-pathogenic, and many have been exploited for biotechnological and industrial purposes ([Raddadi et al. 2012](#_ENREF_14)). Only a few Bacillus species are known to cause sickness in both animals and humans. Bacillus anthracis and Bacillus cereus are regarded medically relevant Bacillus species; B. anthracis is the causative agent of anthrax, a prevalent animal illness, while B. cereus may cause food poisoning as well as local and systemic infections ([Goel, 2015](#_ENREF_8); [Romero-Alvarez et al. 2020](#_ENREF_18)). Escherichia coli belongs to the Enterobacteriaceae family, which consists of Gram-negative facultatively anaerobic rods (with both a fermentative and respiratory metabolism) that do not generate the enzyme oxidase ([Skočková et al. 2021](#_ENREF_21)). It is the leading cause of acute urinary tract infections and urinary tract sepsis. It has also been linked to newborn meningitis, sepsis, and abscesses in a variety of organ systems. *E. coli* may cause acute enteritis in both humans and animals and is a common cause of dysentery-like disease in humans, and haemorrhagic colitis ([Kumari et al. 2020](#_ENREF_11)).In recent years, antimicrobial resistance has become a catastrophic concern for public health. The widespread use of antibiotics in medical, veterinary care, agriculture, and poultry has significantly contributed to the development of bacterial resistance ([Rahman and Sarker, 2020](#_ENREF_15)). Antimicrobial resistance in bacterial pathogens is a problem that has a high morbidity and death rate. Multidrug resistance patterns in Gram-positive and -negative bacteria are difficult to cure, and may be untreatable with standard antibiotics ([Frieri et al. 2017](#_ENREF_7)). However, to overcome this problem of bacterial resistance an alternating potential treatment chosen is the use of herbal medicines ([Boudou et al. 2021](#_ENREF_2)). *Camellia sinensis*, more popularly known as tea, is the world's second most consumed beverage, behind water ([Baills, 2015](#_ENREF_1)). Caffeine and antioxidant polyphenols are found in C. sinensis (green tea). It has been promoted as beneficial in a wide range of illnesses, including cancer prevention, based primarily on limited epidemiological data, cardiovascular problems, and AIDS ([Dubick and Omaye, 2001](#_ENREF_5)). The tea plant *Camellia sinensis* contains several biologically active polyphenols, including catechins such as epigallocatechin-3-gallate (EGCG) and (-)-epigallocatechin (EGC). Green tea consumption has been shown to reduce cardiovascular dysfunction by lowering total and LDL cholesterol, reducing blood pressure ([Liwa et al. 2017](#_ENREF_12)). In this context, and based on this extensive literature on the general health benefits of green tea consumption, the present study aims to determine the phytochemical profiling and the in vitro antibacterial effect of the methanolic extract of *C. sinensis* on two bacterial strains (Gram-positive and Gram-negative), namely: *Bacillus subtilis* ATCC 11778 and *Escherichia coli* ATCC 25922 respectively.

**MATERIALS AND METHODS**

The dried and crushed *C. sinensis* leaves (10 g) were subjected to an extraction by maceration in methanol (100 ml) for 48 hours with renewal of solvent after 24 hours and under magnetic stirring. The macerates were filtered on Whatman No. 1 filter paper and the filtrates were evaporated to dryness using a rotary evaporator ([Ranaweera, 2013](#_ENREF_16)). The extracts obtained were used for phytochemical and antibacterial tests. LC-UV analysis was performed using a Shimadzu Prominence-i LC-2030C 3D apparatus using a C18 Column: 25cm x4.6mm (Supelco), thickness 5 μm at a temperature of 27 °C. The mobile phase consists of a solvent gradient eluent system containing an aqueous solution of acetic acid and methanol at a flow rate of 0.8/min. The analysis of phenol fingerprints was recorded at 280 and 345 nm. Antibacterial activity against *Bacillus subtilis* ATCC 11778 and *Escherichia coli* ATCC 25922 was performed by mean of agar well diffusion method by spreading each bacterial suspension (1.5 x 108 CFU/ml) on the surface of Mueller-Hinton agar plates containing four wells (6 mm Ø) filled with approximately 30 μL of each of the extract concentrations used (625, 1250, 2500, 5000 µg/mL), and a central well filled with methanol (Control). The plates were incubated at 37°C overnight. The results were expressed in terms of the diameter of the inhibition zone and sterile water was used as a control ([Kaushik et al. 2010](#_ENREF_10)). The mean ± SD values were calculated for each group to determine the significance of intergroup differences. To find the difference between the groups, Student’s test was used. P values <0.05 were considered to be significant.

**RESULTS**

The data in Table 1 shows the results of the LC-UV analysis of the methanolic extract obtained by maceration of the leaves of *Camellia sinensis* which reveals the presence of four major compounds, namely: Caffeic acid, Epicatechin, Syringic acid and Epigallocatechin gallate with proportions of 5.12%, 2.39% 1.85% and 15.29 respectively.

Table1. Phytochemical profiling of the methanolic extract of *Camellia sinensis* using LC-UV analysis

|  |  |  |
| --- | --- | --- |
| Compounds | Retention time (min) | Proportion (%) |
| Caffeic acid | 16,70 | 5.12 |
| Epicatechin | 21,53 | 2.39 |
| Syringic acid | 24,44 | 1.85 |
| Epigallocatechin gallate | 34,67 | 1.85 |

The Table 2 and the figure 1 reflect the results of the inhibition tests carried out by agar well diffusion method and showed a strong antibacterial activity the methanolic extract of *Camellia sinensis* at the concentration of 5000 µg/mL with inhibition zones of 25.38±2.75 mm and 22.78±1.22 mm against *Bacillus cereus* ATCC 11778, and *E. coli* ATCC 25922 respectively. whereas at the two concentrations 2500 and 1250 µg /mL the extract showed a moderate activity with inhibition zoning of 15.16±0.95 and 12.84±0.64 respectively for *Bacillus cereus* ATCC and 14.30±1.84 and 9.67±2.50 respectively for *E. coli* ATCC 25922. While, no activity was noted for the last concentration (625 µg /mL) against the two bacterial strains used.

Table 2. The results of the antibacterial activity of the different extract concentrations of *Camellia sinensis* against *Bacillus cereus* ATCC 11778, and *E. coli* ATCC 25922.

|  |  |  |  |
| --- | --- | --- | --- |
| Concentrations (µg/mL) | | | |
| Bacteria | 5000 | 2500 | 1250 | 625 |
| *Bacillus subtilis* | 25.38±2.75 | 15.16±0.95 | 12.84±0.64 | 06.00±0.00 |
| *Escherichia coli* | 22.78±1.22 | 14.30±1.84 | 9.67±2.50 | 06.00±0.00 |

|  |  |
| --- | --- |
|  |  |

Figure 1.  Photographs of antibacterial activity different extract concentrations of *Camellia sinensis* extract and control (methanol) against *Bacillus cereus* ATCC 11778 (A) and *E. coli* ATCC 25922(B).

**DISCUSSION**

Green tea, *Camellia sinensis*, is one of the most popular beverages in the world, second only to water in terms of both enjoyment and health. A substantial amount of study has been conducted to describe the health advantages of green tea for a wide range of issues, including several forms of cancer, heart disease, liver illness, and so on. Green tea can also help with diabetes, exercise enhancement, inflammatory bowel disease, skin diseases, hair loss, weight reduction, and iron overload ([Sinija and Mishra, 2008](#_ENREF_20)). On the other hand, the world is witnessing the emergence of a major public health problem related to bacterial resistance to antibiotics ([VT Nair et al. 2018](#_ENREF_22); [Pulingam et al. 2021](#_ENREF_13)). It is for this reason that it is urgent to find new natural molecules with antibacterial effects to face the problem of antibiotic resistance ([Das and Satyaprakash, 2018](#_ENREF_4)). In this context, the present study aims to determine the phytochemical profiling and the in vitro antibacterial effect of the methanolic extract of *Camellia sinensis* on two bacterial strains (Gram-positive and Gram-negative), namely: Bacillus subtilis ATCC 11778 and Escherichia coli ATCC 25922 respectively. Indeed, the results of the LC-UV analysis of the methanolic extract obtained by maceration of Camellia sinensis leaves revealed the presence of four major compounds, namely : Caffeic acid, epicatechin, syringic acid and epigallocatechin gallate with proportions of 5.12%, 2.39% 1.85% and 15.29 respectively. in effect, a previous study confirms our data and indicates that the Camellia sinensis tea plant contains several biologically active polyphenols, including catechins such as epigallocatechin-3-gallate (EGCG) and (-)-epigallocatechin (EGC) ([Liwa et al. 2017](#_ENREF_12)). In addition, a study to evaluate the phytochemical profile and antioxidant properties of Italian green tea indicates that HPLC analysis identified a total of thirteen phenolic compounds including catechins, benzoic acids, cinnamic acids, and flavonols ([Falla et al. 2021](#_ENREF_6)). While, the inhibition tests performed by agar well diffusion method showed strong antibacterial activity of methanolic extract of Camellia sinensis at the concentration of 5000 µg/mL with inhibition zones of 25.38±2.75 mm and 22.78±1.22 mm against Bacillus cereus ATCC 11778, and E. coli ATCC 25922 respectively. Identical results were reported in a study done on green tea extract to identify the possible mechanism and antibacterial activity on skin pathogens, shows that aqueous extract of green tea leaves possess antibacterial effects on selected bacterial strains, namely: Staphylococcus epidermidis, Micrococcus luteus, Brevibacterium linens, Pseudomonas fluorescens and Bacillus subtilis which were found to be sensitive to green tea extract by disc diffusion test (inhibition zone ⩾7 mm) ([Sharma et al. 2012](#_ENREF_19)). In order to better understand the mechanism of antimicrobial activity of plant extracts, changes in internal pH (pHint) and membrane potential were measured in Staphylococcus aureus (SA) and Escherichia coli (EC) cells after exposure to plant extracts. The results indicate that plant extracts significantly affect the cell membrane of both Gram-positive and Gram-negative bacteria, as evidenced by a decrease in pHint as well as hyperpolarisation of the cell membrane ([Gonelimali et al. 2018](#_ENREF_9)). Green tea catechins' antibacterial activity is mediated by a number of mechanisms, which can be broadly classified as follows: (1) inhibition of virulence factors (toxins and extracellular matrix); (2) cell wall and membrane disruption; (3) inhibition of intracellular enzymes; (4) oxidative stress; (5) DNA damage; and (6) iron chelation. These systems work in tandem, and the relative relevance of each varies between bacterial strains. Galloylated chemicals have the highest antibacterial activity in all investigations (EGCG, ECG and theaflavin digallate) ([Renzetti et al. 2020](#_ENREF_17)). Another study summarizes the proposed antibacterial mechanisms of tea flavonoids as follows: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of attachment and biofilm formation, inhibition of porin on the cell membrane, alteration of membrane permeability and attenuation of pathogenicity ([Xie et al. 2015](#_ENREF_23)).

**CONCLUSION**

The study's findings revealed that the extract obtained from green tea, *Camellia sinensis*, by maceration with methanol as a solvent had strong antibacterial activity against *Bacillus cereus* ATCC 11778 and *E. coli* ATCC 25922, whose mechanism of action is correlated with its high content of phenolic compounds identified by LC-UV analysis, namely Caffeic acid, Epicatechin, Syringic acid, and Epigallocatechin gallate. These findings show that *C. sinensis* can be exploited to identify novel natural medicinal compounds to treat pathogenic bacteria and overcome the problem of bacterial resistance to synthetic antibiotics.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**REFERENCES**

Baills T. (2015). "The Consumer behavior behind the most consumed beverage in the world, Camellia sinensis: case study on French consumers."

Boudou F, Belakredar A, Zaoui O, Sehmi, A., Ghomid K. (2021). Phytochemical, antioxidant, and antibacterial properties of Camellia sinensis. Journal of Experimental Research 9(4).

Cote CK, Heffron JD, Bozue JA, & Welkos SL. (2015). Bacillus anthracis and other Bacillus species. Molecular medical microbiology, Elsevier: 1789-1844.

Das A and Satyaprakash K. (2018). Antimicrobial properties of natural products: A review. Pharma. Innov. J 7: 532-537.

Dubick M and Omaye ST. (2001). Modification of atherogenesis and heart disease by grape wine and tea polyphenols. Nutraceuticals Handbook: 235-260.

Falla NM, Demasi S, Caser M, Scariot V.  (2021). Phytochemical profile and antioxidant properties of italian green tea, a new high quality niche product. Horticulturae 7(5): 91.

Frieri M, Kumar K, Boutin A. (2017). Antibiotic resistance. Journal of infection and public health 10(4): 369-378.

Goel AK. (2015). Anthrax: A disease of biowarfare and public health importance. World Journal of Clinical Cases: WJCC 3(1): 20.

Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontiers in microbiology 9: 1639.

Kaushik P, Goyal P, Chauhan A, Chauhan G. (2010). In vitro evaluation of antibacterial potential of dry fruitextracts of Elettaria cardamomum Maton (Chhoti Elaichi). Iranian journal of pharmaceutical research: IJPR 9(3): 287.

Kumari A, Kumar P, Kumar M, Kumar J.  (2020). Antibacterial Activity of Glycyrhhiza Glabra Root Extracts against Staphylococccus Sp and Escherichia Coli. Kumari, A., Kumar, P., Kumar, M. and Kumar, J.

Liwa AC, Barton EN, Cole WC, Nwokocha CR. (2017). Bioactive plant molecules, sources and mechanism of action in the treatment of cardiovascular disease. Pharmacognosy, Elsevier: 315-336.

Pulingam T, Parumasivam T, Gazzali AM, Sulaiman AM, Chee JY, Lakshmanan M, Sudesh K. (2021). Antimicrobial resistance: Prevalence, Economic Burden, Mechanisms of Resistance and Strategies to Overcome.European Journal of Pharmaceutical Sciences: 106103.

Raddadi N, Crotti E, Rolli E, Marasco R, Fava F, Daffonchio D. (2012). The most important Bacillus species in biotechnology. Bacillus thuringiensis biotechnology, Springer: 329-345.

Rahman M and Sarker SD. (2020). Antimicrobial natural products. Annual Reports in Medicinal Chemistry, Elsevier. 55: 77-113.

Ranaweera S. (2013). Mosquito-lavicidal activity of some Sri Lankan plants. Journal of the National Science Foundation of Sri Lanka 24(2).

Renzetti A, Betts JW, Fukumoto K, Rutherford RN. (2020). Antibacterial green tea catechins from a molecular perspective: Mechanisms of action and structure–activity relationships. Food & function 11(11): 9370-9396.

Romero-Alvarez D, Peterson AT, Salzer JS, Pittiglio C, Shadomy S, Traxler R, Campbell LP. (2020). Potential distributions of Bacillus anthracis and Bacillus cereus biovar anthracis causing anthrax in Africa. PLoS neglected tropical diseases 14(3): e0008131.

Sharma A, Gupta S, Sarethy IP, Dang S, Gabrani R. (2012). Green tea extract: possible mechanism and antibacterial activity on skin pathogens. Food chemistry 135(2): 672-675.

Sinija V and Mishra HN. (2008). Green tea: Health benefits. Journal of Nutritional & Environmental Medicine 17(4): 232-242.

Skočková A, Cupáková Š, Karpíšková R, Janštová B. (2021). Detection of tetracycline resistance genes in Escherichia coli from raw cow’s milk. Journal of Microbiology, Biotechnology and Food Sciences 2021: 777-784.

VT Nair D, Venkitanarayanan K, Kollanoor Johny A. (2018). Antibiotic-resistant Salmonella in the food supply and the potential role of antibiotic alternatives for control. Foods 7(10): 167.

Xie Y, Yang W, Tang F, Chen X, Ren L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current medicinal chemistry 22(1): 132-149.