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PHYTOCHEMICAL, ANTIOXIDANT, AND ANTIBACTERIAL PROPERTIES OF *CAMELLIA SINENSIS*

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ABSTRACT

Like many other aromatic plants,green tea (*Camellia sinensis*),synthesize some benefic bioactive molecules that can be used to cure certain diseases. This study aimed to evaluate the phytochemical composition, the antioxidant potential, and the *in vitro* antibacterial activity of *C. sinensis* infusion against two pathogenic bacteria (*Enterobacter cloacae* and *Listeria monocytogéne)*. The phytochemical screening revealed the presence of flavonoids, tannin, ellagic acid, proanthocyanidins, saponins, and polyphenols. Total polyphenol estimation reveals a level of 213±10 mg AGE/g. While the antioxidant activity evaluation reveals a significant ability to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical ($IC_{50\%}$=5.57±0.08 mg/mL). The inhibition tests carried out by the disk diffusion and agarwell diffusion methods showed a strong antibacterial activity with diameters varying from 16.33±0.45 to 22.67±0.44 mm using the agar well method and from 10.00±0.44 to 12.33±0.89 mm using the disk method against *E. cloacae* and *L. monocytogéne* respectively. This study allowed us to conclude on the effectiveness of *Camellia sinensis* against these two pathogenic bacteria.

Keywords: Antibacterial, antioxidant, DPPH, Polyphenol, *Tea*.

INTRODUCTION

Since the advent of antibiotics, there has been a clear improvement in quality and life expectancy. But, their intensive use resulted in the adaptation of bacteria to these remarkable substances. Indeed, in recent years there has been a disturbing increase in the multi-resistance of pathogenic bacteria, the resurgence of infectious diseases, which were believed to be perfectly controlled, and the emergence of new pathogens, especially in developing countries (Thangapazham et al. 2007; Fair and Tor, 2014). *Listeria monocytogenes* a gram-positive bacterium is salt resistant and highly adapted. This organism is motile, psychrophilic, and occurs everywhere in the environment. It is isolated from the silo, vegetable, dairy foods, red meat, ready-to-eat food products, etc. It causes listeriosis in humans, other animals, and birds. The organism is recognized as a food-borne pathogen (Ushimaru et al. 2007). *Enterobacter cloacae* is a member of the normal gut flora of many humans where some strains have been associated with urinary tract and respiratory tract infections in immunocompromised individuals (Kaneria et al. 2009; Samba et al. 2015). However, to overcome this problem an alternative potential treatment chosen is the use of herbal medicines as *Camellia sinensis* infusion, commonly known as tea, which is the second most commonly drank liquid on earth after water and is being consumed socially and habitually by people since 3000 BC (Nair, 2021). There are two major kinds of tea, black tea, and green tea, and they both contain caffeine (1–5%) with small amounts of other xanthine alkaloids also present. Tea composition varies with climate, season, tea variety, and age of the leaf. Tea also contains large amounts of tannins or phenolic substances (5–27%) consisting of catechin (flavanol) and gallic acid units, with those in green tea is higher than those in black tea (Hayouni et al. 2007). The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin, and polyphenols (Ushimaru et al. 2007). Phenolicscan act as antioxidants in several ways. These antioxidants act as reducing agents, hydrogen donators, free radicals scavengers, and singlet oxygen quenchers and, therefore, as cell saviors (Ekayanti et al. 2017). Several polyphenolic compounds extracted from green tea, especially catechins are potent antioxidants and to be effective in preventing cancer (Kim, 2013). Therefore, the current study aims to evaluate the phytochemical composition, and the antioxidant, and the *in vitro* antibacterial activities of *C. sinensis*infusion against these two pathogenic bacteria.

MATERIALS AND METHODS

Preparation of plant extract

The infusion was prepared by taking 10 g of dry leaves of *C. sinensis*dissolved in 100 mL of preheated distilled water and the mixture is stirred for 10 minutes. The solution obtained is filtered on Wattman Millipore filter paper and the filtrate is sterilized by injection under a micro-filter (Retention threshold: 0.45 µm) (Tariq and Reyaz, 2013).

Phytochemical and antioxidant estimations

Phytochemical tests were performed on the *C. sinensis'* infusion to detect secondary metabolites: flavonoids, tannin, ellagic acid, proanthocyanidins, saponins and polyphenols, according to the experimental procedures described by Samba et al. ,2015 (Samba et al. 2015). The result of each test is qualitatively and phytochemically expressed by the sign (+) presence and (-) absence. While, the determination of the polyphenols was carried out according to the method described by Vermerius and Nicholson, (2008) using the Folin-Ciocalteu reagent and referring to a calibration curve of Gallic acid.The scavenging ability of tea samples on DPPH radical was measured according to the method reported by Santos and Gonçalves, (2016). The positive control is represented by a solution of ascorbic acid. The inhibitory concentration 50 (IC50) values were calculated from data obtained graphically.

Antibacterial activity

The inoculum was prepared by culturing the microorganisms in nutrient broth at 37°C for 12 hours and a concentration of approximately 10^5 CFU/mL was used for the antimicrobial analysis (Hayouni et al. 2007). The Agar-well diffusion method was performed by spreading each bacterial suspension over the surface of Mueller-Hinton agar plates containing a central well having 6 mm diameter. The well was filled with 30 µL of extract. While disc diffusion method was performed using discs of filter paper, 6 mm diameter, impregnated with about 30 µL of the sterile extract and deposited in the center of the previously prepared Petri dishes. Other disks impregnated only with sterile distilled water are used as a control test. The plates were incubated at 37°C for overnight. The results were expressed in terms of the diameter of the inhibition zone and sterile water used as control (Kaushik et al. 2010).

Statistical analysis

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 t-test was used. P values <0.05 were considered to be significant.

RESULTS

The results of phytochemical tests performed on the aqueous extract of *C. sinensis* are grouped in Table 1. These tests consist in detecting the different families of secondary metabolites existing in the extract by qualitative reactions of characterization. These reactions are based on phenomena of precipitation or coloration by reagents specific to each family of compounds.

Table 1: Different groups of secondary metabolites identified in *Camellia sinensis*

phytochemical compounds	Flavonoids	Tannin	Ellagic acid	Proanthocyanidols	Polyphenols	Saponins
Observations	+	+	+	+	+	+

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Determination of total phenol contents in the aqueous extract of tea using the Folin-Ciocalteux colorimetric method was reported in mg gallic acid equivalent (AGE)/g dry plant material according to the calibration curve (figure 1). The results show that the aqueous extract has a high content of total phenols (213±10 mg AGE/g).

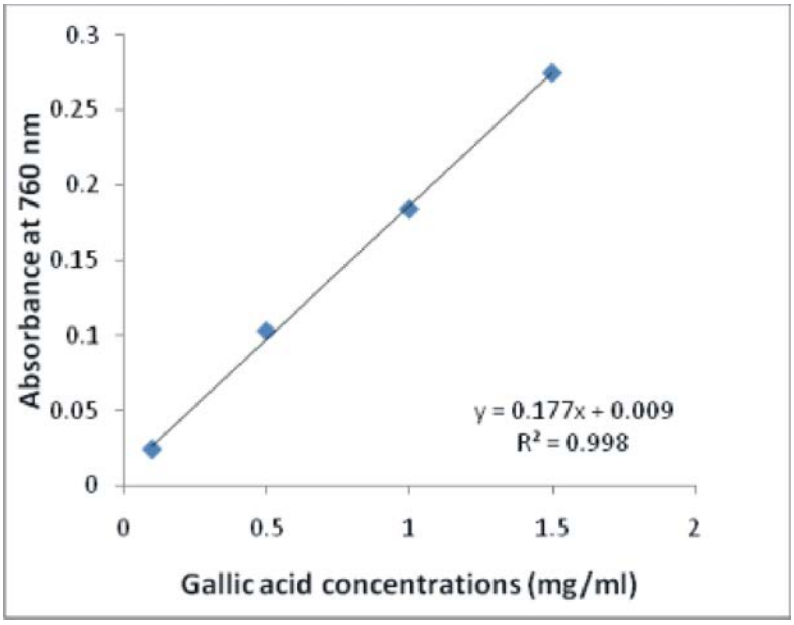

Figure 1. Gallic acid calibration curve.

The antioxidant activity of the aqueous extract and the standard antioxidant (ascorbic acid) towards the DPPH radical was evaluated with a spectrophotometer by following the reduction of this radical which is accompanied by its passage of the purple color (DPPH-) to the yellow color (DPPH-H) measurable at 515 nm. According to the results recorded, the aqueous extract is endowed with an important antioxidant power with an IC50 value of 5.57±0.08 mg/mL.However, when compared to ascorbic acid, it remains significantly lower ($p<0.05$), especially in the first two concentrations (figure 2).

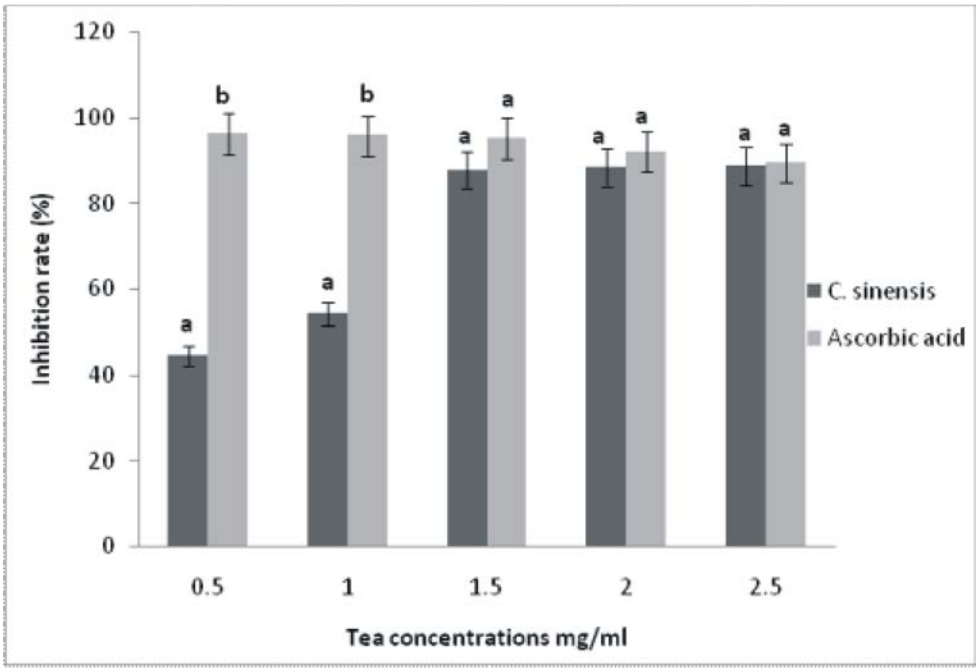

Figure 2. Results of DPPH inhibition test of *C. sinensis'* infusion, and ascorbic acid.

Data are expressed as means ± SD (n=6). A comparison between groups was made using the student's t-test. Bars not sharing a common letter (a–c) differ significantly at p < 0.05.

Figures 3 and 4 reflect the results of the inhibition tests carried out by the disk diffusion and agarwell diffusion methods and showed a strong antibacterial activity with diameters varying from 16.33±0.45 to 22.67±0.44 mm using the agar well method and from 10.00±0.44 to 12.33±0.89 mm using the disk diffusion method against *E.cloacae* and *L. monocytogéne* respectively. This implies that the tea extract's antibacterial action is important against both bacteria strains. It is, however, significantly far more efficient against gram-positive bacteria than gram-negative bacteria.

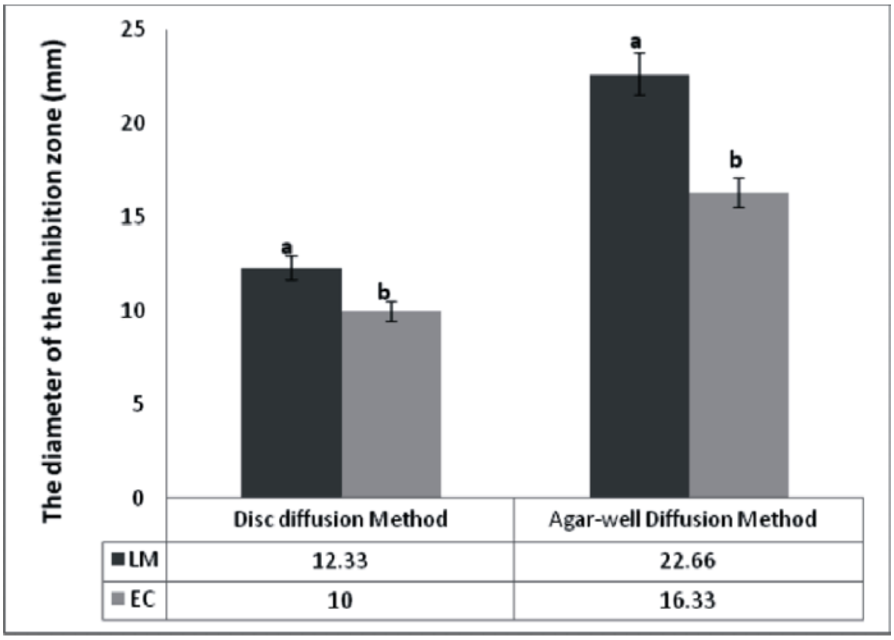

Figure 3.Results of the inhibition zones of the antibacterial activity of *C. sinensis'* infusion against Listeria monocytogéne (LM), Enterobacter cloacae (EC).

Data are expressed as means ± SD (n=6). A comparison between groups was made using the student's t -test. Bars not sharing a common letter (a–c) differ

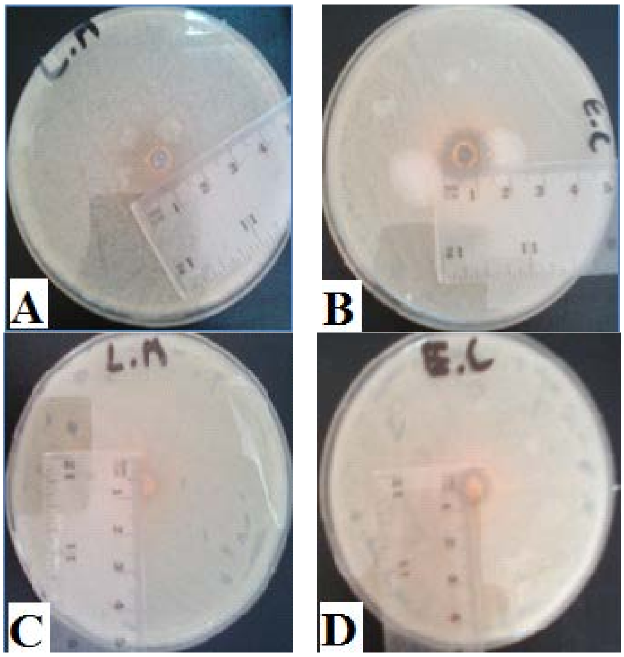

Figure 4.Photographs of results of the antibacterial activity by Agar-well diffusion, and disk methods.

***A** & **B**: show the inhibition zones of tea extract tested on Listeria monocytogenes (LM) andEnterobacter cloacae (EC). respectively by agar well method. **C&D**: shows the inhibition zones of the tea extract tested on LM and EC respectively by the disc method.*

DISCUSSION

Results of the current study revealed that the aqueous extract of *C. sinensis*is rich in secondary metabolites namely: flavonoids, tannin, ellagic acid, proanthocyanidins, saponins, and polyphenols highlighted by qualitative phytochemical tests based on precipitation reactions or staining by reagents specific to each family of compounds.These results are in agreement with those of Dogra et al. (2011), who indicated that the aqueous extract of Indian green tea is rich in tannins, flavonoids, terpenoids, glycosides, alkaloids, steroids, saponins, and anthraquinones.In addition,Inamdar et al. (2014), demonstrated that *Camellia sinensis* is a rich source of flavonoid compounds. The quantitative analysis of polyphenols by a spectrophotometric method using Gallic acid as standard in the calibration curve revealed that the aqueous extract obtained by infusion of *Camellia sinensis* has a significant amount of phenolic compounds estimated 213±10 mg AGE/g. The same phenolic content value of 243 ± 70 mg GAE/g dry weight was reported by Luo et al. (2020) obtained by green extraction using ultrasound. While ethanol was the best solvent system for extracting total polyphenols from C. sinensis (355.26 ± 23.68 AGE/g) (Gadkari et al. 2014). However, the use of a green tea infusion has shown good results with the two methods of inhibition used against *Enterobacter cloacae and listeria monocytogenes*. These results are confirmed by several other works which indicate in the first place, that green tea has the qualities of its very rich chemical composition (alkaloids, flavonoids, steroids, gallic tannins, catechol tannin) (Chakraborty et al. 2014; Parajuli et al. 2020). The highest antimicrobial activity of tea is due to presence of catechins and polyphones which damages bacterial cell membrane (Taylor et al. 2005; Wu and Brown, 2021). Indeed, our study shows that green tea is rich in secondary metabolites like flavonoids, Tannin, Ellagic acid, proanthocyanidins, polyphenols and saponins. Other works shows that the méthanolic extract had a great antibacterial property by disc diffusion method compared to the water extract (Mbata et al. 2008). Inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of attachment and biofilm formation, inhibition of the porin on the cell membrane, alteration of membrane permeability, and attenuation of pathogenicity are some of the proposed antibacterial mechanisms of flavonoids (Xie et al. 2015). Antimicrobial agents are classified depending on their antimicrobial action mechanism. Inhibitors of cell wall production, depolarizers of the cell membrane, inhibitors of protein synthesis, inhibitors of nucleic acid synthesis, and inhibitors of metabolic pathways in bacteria are the primary categories (Reygaert, 2018). Methanolic extract of *Camellia sinensis*had also a greater antibacterial activity against *Staphylococcus aureus*and *Escherichia coli* (Chan et al. 2011). While, several substances including antibiotics have difficulty penetrating the walls of Gram-negative (Smith and Dou, 2001; Kaneria et al. 2009). Our results indicate that not only the green tea has a great effect on Gram-positive bacteria but also on Gram-negative.

CONCLUSION

Camellia sinensis leaves are having a dual benefits as medicinal values and food value. In this study, we found that leaf extracts are rich in secondary metabolites and were found to be potential antibacterial agents against the gram-positive, but also on the Gram-negative which pose resistance to several other substances. Thus *C.sinensis* leaves can be used as alternative medicine against the bacterial infection.

Conflicts of Interest

Authors have declared that they have no competing interests.

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