

Evaluation of Antimicrobial Activity of Aqueous Extract of White Tea *Camellia sinensis* L. Kuntze (1887)

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Abstract: Background: The present study aimed to investigate the potential antimicrobial activity of aqueous extracts of white tea (*Camellia sinensis*), which were obtained by infusing and decocting. The disk-agar diffusion assay was performed using disks saturated with aqueous solutions at concentrations of 1 mg mL⁻¹ to 20 mg mL⁻¹, against bacteria as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. **Results:** Growth inhibition of *S. aureus* was observed in decocts at 10 mg mL⁻¹ concentration. **Conclusion:** Results obtained in this work suggested a potential antimicrobial activity of white tea against gram positive bacteria.

Key words: Antimicrobial, aqueous, *Camellia sinensis*, extract, white tea

INTRODUCTION

Tea is one of the most popular beverages worldwide and the white tea is obtained from the leaves of *Camellia sinensis* L. Kuntze 1887. This product has been consumed since ancient times and there are reports of its use since Shen Nung dynasty, in 2300 B.C., in China (Simões *et al.*, 2004). This species of plants belongs to the family Theaceae (Linnaeus) and several types of tea are produced from this plant, whose differences in aroma and flavor are related to the process of tea preparation employed. The most common types of tea produced include green, black, oolong and white tea (Simões *et al.*, 2004; Hsu, 2005; Friedman *et al.*, 2007).

Currently, a growing consumption of tea is observed in western countries, where it has been considered as functional food. The biological properties of tea include effects on the Central Nervous System (CNS) and antioxidant effects, attributed to the presence of methylxanthines, such as caffeine and phenolic compounds, especially catechins (Pasha and Reddy, 2005; Chen *et al.*, 2007; Reto *et al.*, 2008).

Among recent studies, a significant increase has been observed in the number of investigations on species of plants with potential antimicrobial activity, due to the growing problem associated with bacterial resistance against the available therapeutic arsenal (Koo *et al.*, 1999).

Only a few studies, however, focus on the evaluation of antimicrobial activity of white tea and caffeine, although several scientific studies are related to green tea and catechins (Sharangi, 2009).

Within this context, the present study aimed to investigate the antimicrobial action of aqueous extracts of white tea, against bacteria as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and caffeine, at concentrations of 0.01 to 0.2 mg mL⁻¹, as well as to determine this substance in the sample analyzed using the modified Stahl's method.

MATERIALS AND METHODS

Tea specimens: Tea specimens were purchased from a pharmacy in the city of Novo Hamburgo, Southern Brazil, as tea bags stored in paper boxes, properly identified.

Determination of caffeine: Determination of caffeine in tea specimens was based on a standard curve generated with the respective reference substance, which was later quantified in the sample using the Modified Stahl's technique-MS.

Standard curve for caffeine: For the standard curve, an exact dose of 5 mg of caffeine (reference substance-Merck) was weighed, dissolved in 10 mL distilled water,

transferred quantitatively to a 50 mL volumetric flask and then diluted to volume with the same solvent. Volumes of 0.15, 0.25, 0.50, 1.0, 1.5, 2.0, 2.5 and 3.0 mL of the above solution were pipetted into 10 mL volumetric flasks and diluted to volume with water. The absorbance of each solution was measured at 273 nm using a Dig Varian Cary UV visible spectrophotometer (serial no.: 50NSEL03127475; Varian Inc., Palo Alto, California, USA) and readings were performed in triplicate (Baldo, 1992).

Modified stahl's technique-MS: An exact dose of 1 g of tea sample was extracted with 15 mL sulfuric acid (1 M), heated for 15 min in a double boiler. After the extract was filtered, the residue was washed with 10 mL distilled water. The filtrate was then alkalized with ammonium hydroxide (P.A. Merck), to pH 10 and transferred quantitatively to a separatory funnel. This solution was extracted with 3 times 10 mL chloroform (P.A. Merck). Chloroform phases were placed into 50 mL Erlenmeyer flasks and dried over anhydrous sodium sulfate. The chloroform solution, with additional 2 mL chloroform used to rinse the Erlenmeyer flask, was transferred to a porcelain crucible and evaporated to dryness (37°C) in a double boiler. The residue was dissolved in distilled water, transferred quantitatively to a 100 mL volumetric flask and diluted to volume with distilled water. A volume of 1 mL of this solution was transferred to a 10 mL flask and diluted to volume with distilled water. The absorbance of the sample was measured at 273 nm in the UV visible spectrophotometer. Three readings were performed for each sample assayed and the arithmetic means were then calculated (Baldo, 1992).

Microbiological assays

Preparation of aqueous extracts of white tea: To obtain the aqueous extracts used in the microbiological assays, tea specimens were taken from the tea bags and the parts not properly rubbed were pounded with a pestle and mortar. To standardize granulometry, we followed the recommendations in the 4th edition of the Brazilian Official Pharmacopoeia concerning plant use (Oga and Zanini, 1988).

Infusion was prepared by pouring boiling water on samples, covering the container; samples were allowed to rest for 15 min, with subsequent filtration. Regarding decoct, samples were steeped in boiling water (100°C) for 15 min, with subsequent filtration (Schuck *et al.*, 2001). Final concentrations of these aqueous extracts were: 1, 5, 10, 15 and 20 mg mL⁻¹ (p:V).

Preparation of caffeine solutions: In order to verify the antimicrobial activity of caffeine, a mother-solution was

prepared from an exact dose of 5 mg of caffeine (reference substance-Merck), which was dissolved in 10 mL distilled water, transferred quantitatively to a 50 mL volumetric flask and then diluted to volume. Volumes of 0.1, 0.2, 0.5, 1.0 and 2.0 mL of this solution were pipetted into 10 mL volumetric flasks and diluted to volume with distilled water, resulting in 0.01, 0.02, 0.05, 0.1 and 0.2 mg mL⁻¹ concentrations.

Evaluation of antimicrobial activity: The evaluation of antimicrobial activity was performed by the disk diffusion method, according to the technique described by Berghe and Vlietnck (1991).

The samples of white tea and caffeine aqueous extracts were sterilized using the vacuum filtration system (0.22 µm). Petri plates with Mueller-Hinton agar (4 mm height) were previously prepared and each plate was inoculated with 0.1 mL standard bacterial strains (ATCC) adjusted to 0.5 McFarland scale. The sterile paper disks were placed on the culture medium and 20 µL of each sample was added to each disk. Plates were then incubated at 35°C for 18 to 20 h and inhibition zone diameters were measured. Each concentration of white tea and caffeine was assayed in triplicate and results were expressed as millimeters by the mean inhibition zone diameter formed around the disks. Positive-control testing was performed with disks containing chloramphenicol (30 µg) and amikacin (30 µg) (Sygma).

Determination of Minimum Inhibitory Concentration

(MIC): MIC was tested by serial dilution assay in samples in which growth was positive. The samples were incorporated into 0.5 mL Mueller-Hinton broth, at 0.625, 1.25, 2.5, 5 and 10 mg mL⁻¹ (p:V) concentrations for tea extracts and 0.00625, 0.0125, 0.025, 0.05 and 0.1 mg mL⁻¹ (p:V) concentrations for caffeine solutions. A 0.5 mL suspension of microorganisms was transferred to each tube. There was one negative control tube, containing only microorganisms without the samples and a positive control tube, containing only chloramphenicol (2 mg mL⁻¹). The tubes were incubated at 37°C for 24 h. The lowest concentrations, which did not show macroscopic growth of the microorganism assayed, were classified as MIC.

Statistical analysis: Statistical analysis was performed using Analysis of Variance (ANOVA), followed by Tukey's test and the SPSS software for Windows. The correlation coefficient was determined through analysis in the Excel 2007 software.

RESULTS AND DISCUSSION

Second only to water, tea is considered the most widely consumed beverage in the world today, not only

due to its pleasant flavor and aroma, but also because tea consumption has beneficial effects on health (Reto *et al.*, 2008).

Currently, in scientific research, a growing trend is observed toward studies analyzing functional food, which, in addition to a food source, part of them or isolated substances, have had their pharmacological activity demonstrated as capable of promoting health benefits, in the prevention and/or treatment of diseases (Moraes and Colla, 2006). Among the species of main interest, *C. sinensis* stands out because of its potential antioxidant properties, related mainly to catechins, in addition to providing an important source of flavonoids (Pasha and Reddy, 2005; Chen *et al.*, 2007).

The chemical structure of *C. sinensis* is mainly composed of polyphenolic compounds, such as catechins and flavonoids, alkaloids, with special emphasis on caffeine and theophylline, volatile oils, polysaccharides, amino acids, lipids, vitamins, inorganic elements, such as aluminum, fluorine, manganese, among others (Hakim *et al.*, 2003; Lago, 2007; Sharangi, 2009). A preliminary phytochemical analysis corroborated these data, since the presence of phenolic compounds, flavonoids, tannins, alkaloids and steroid and triterpenoid nuclei was determined in the sample analyzed.

Regarding data on determination of caffeine, the results obtained in the standard curve for caffeine exhibited good linearity, with a correlation coefficient of $r^2 = 0.9999$, described by the equation $y = 31.236x - 0.0511$ (Fig. 1).

Caffeine, the main alkaloid present in *C. sinensis*, accounts for approximately 2% of the plant composition (Ashihara *et al.*, 2008). In this study, the mean concentration obtained was 0.94% (Table 1), a value below the mean for this plant species. It is worth noting that chemical composition depends on several factors related to leaf age, climate (humidity, temperature, latitude), cultivation conditions (soil, water, use of fertilizers, among others) and the harvest season (Horzic *et al.*, 2009). Corroborating these observations, Ashihara *et al.* (2008) verified that caffeine synthesis was more pronounced in leaves harvested during spring, due to an increased expression of the TSC1 gene, which codifies caffeine synthesis. Another factor that might have contributed to the low performance of this substance is the method for manufacturing the plant sample, since losses may occur during processing, once caffeine may suffer sublimation during the process of drying leaves at high temperatures (Alves and Bragagnolo, 2002; Ashihara *et al.*, 2008).

White tea differs from others regarding the processing method: white tea is obtained from young

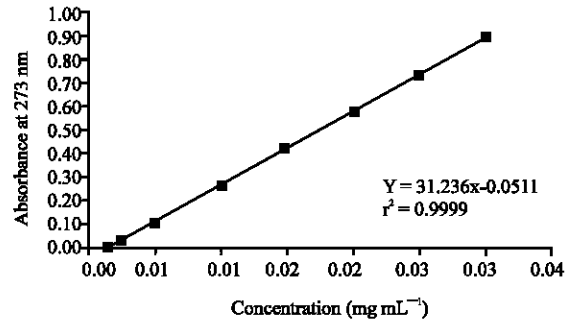


Fig. 1: Standard curve for caffeine in absorbance at 273 nm

Table 1: Caffeine concentration of white tea

Sample/white tea	MS absorbance	Concentration (mg mL ⁻¹)	%
Reading 1	0.2410	0.0094	0.94
Reading 2	0.2680	0.0102	1.02
Reading 3	0.2150	0.0085	0.85
Mean	0.2413	0.0094	0.94

MS = Modified Stahl's technique

leaves, even before buds are completely opened. These whitish leaves are then steamed and dried in the sun, but not fermented, thus becoming a more rare and expensive tea (Friedman *et al.*, 2007; Muthumani and Kumar, 2007; Sharangi, 2009).

Since white tea is a plant of great medicinal relevance and due to the increased consumption of the tea obtained by this processing method, we investigated the potential antimicrobial activity of aqueous extracts of white tea.

Antimicrobial activity was verified only in samples of aqueous extract obtained by decocting, at 5 to 20 mg mL⁻¹ concentrations, in which inhibition zone diameters were observed against *S. aureus* ATCC 25923, with no significant differences among them ($p > 0.05$). The positive controls chloramphenicol and amikacin promoted inhibition zone diameters against gram-negative bacteria and staphylococci, revealing good antimicrobial performance (Table 2).

Regarding the positive antimicrobial evaluation of *S. aureus*, it was submitted to Minimum Inhibitory Concentration (MIC), which corresponds to the lowest concentration for the occurrence of bacterial growth inhibition (Table 3, 4). Serial dilutions revealed inhibitory action starting at 10 mg mL⁻¹ concentration for this type of microorganism.

Caffeine (1,3,7-trimethylxanthine) is one of the main bioactive compounds found in *C. sinensis*. It is an alkaloid with psychoactive components, considered as a mild stimulant, whose effect on human behavior has been an object of study for several years (De Maria and Moreira, 2007).

Table 2: Evaluation of antimicrobial activity by disk diffusion

Bacteria	Concentration (mg mL ⁻¹)	Mean inhibition zone diameter (mm)		Concentration (mg mL ⁻¹)	Caffeine Mean inhibition zone diameter (mm)
		White tea/Decoct	White tea/Infusion		
<i>P. aeruginosa</i>	1	-	-	0.01	-
	5	-	-	0.02	-
	10	-	-	0.05	-
	15	-	-	0.10	-
	20	-	-	0.20	-
Amikacin control (30 µg)		27 mm	25 mm		27 mm
<i>E. coli</i>	1	-	-	0.01	-
	5	-	-	0.02	-
	10	-	-	0.05	-
	15	-	-	0.10	-
	20	-	-	0.20	-
Chloramphenicol control (30 µg)		26 mm	26 mm		26 mm
<i>S. aureus</i>	1	-	-	0.01	-
	5	8.33	-	0.02	-
	10	9.33	-	0.05	-
	15	10.00	-	0.10	-
	20	9.66	-	0.20	-
Chloramphenicol control (30 µg)	-	26 mm	26 mm	-	29 mm

-: Without inhibition zone

Table 3: Minimum Inhibitory Concentration (MIC) of aqueous extract (decoct) of white tea

Aqueous extract of white tea (mg mL ⁻¹)	<i>S. aureus</i>
0.625	+
1.250	+
2.500	+
5.000	+
10.00	-

+: Growth, -: Inhibition

Table 4: Minimum Inhibitory Concentration (MIC) of caffeine solution

Caffeine solution concentration (mg mL ⁻¹)	<i>S. aureus</i>
6.25×10 ⁻³	+
12.5×10 ⁻³	+
25.0×10 ⁻³	+
50.0×10 ⁻³	+
100.0×10 ⁻³	+

+:Growth

In addition to this pharmacological action on the CNS, there are several studies concerning the investigation of potential biological activities of this substance (Kim and Sano, 2008). Among these, some studies have reported an antiviral activity of caffeine. This activity was observed in a study conducted by Utsunomiya *et al.* (2008), in which the authors verified inhibition of viral multiplication against herpes simplex virus type 1 (HSV-1) at a concentration of 5 mM.

In a study conducted by Kim and Sano (2008), caffeine showed antibacterial activity, at 1 mM concentration, against *Pseudomonas syringae* strains, a species of bacteria which can infect plants, such as *Nicotiana tabacum*. Against fecal coliforms, caffeine at 0.5 and 5.0 mg mL⁻¹ concentrations inhibited bacterial growth of *Salmonella typhimurium* (Wu *et al.*, 2007).

Previous studies reported that caffeine showed significant antimicrobial activity, possibly due to free

radical scavenging capacities, thus inhibiting the bacterial oxidation system (Sabu *et al.*, 2002; Azam *et al.*, 2003; Frei and Higdon, 2003; Jardim, 2005; Ashihara *et al.*, 2008). In the present study, antimicrobial activity of this substance was not verified against the bacteria analyzed (Table 2, 4).

Our results indicate that the potential antimicrobial activity observed was not due to the presence of caffeine, since, against the reference substance, it did not show a bacterial growth inhibition zone diameter. We suggest that antimicrobial activity be attributed to the presence of catechins, since white tea shows a significant content of these polyphenols, ranging from 45-90% (Sharangi, 2009).

There are numerous studies on these polyphenolic derivatives, to which a wide range of biological activities have been attributed (Koutelidakis *et al.*, 2009). Almajano *et al.* (2008) analyzed antimicrobial activity of green tea and attributed such action to the presence of catechins, at 1.5% concentration (p:V). Polyphenols and catechins of green tea are considered to scavenge free radicals and to chelate metal ions, being used as natural antioxidants, antibacterial agents and antiviral agents (Tang *et al.*, 2001; Yilmaz, 2006).

The antimicrobial effect observed in the present study may also be attributed to a synergistic action of phytotherapeutic compositions, or isolated from some secondary metabolites, which could have an effect on the synthesis of the peptidoglycan wall, an important component of gram-positive bacteria, thus promoting antibacterial activity.

Unlike antibiotic and chemotherapeutic agents, there are few reports in the literature concerning the possible mechanism of action of plant-derived products. Such

compounds may act on the intermediary metabolism by activating enzymes, altering the action of inhibitors which affect nutrients in the medium, interfering with enzymatic processes at the level of the nucleus or ribosome, causing changes in membranes, or even interfering with secondary metabolism (Cowan, 1999).

Since medicinal plants produce a variety of substances with antimicrobial properties, screening programs are expected to find out new compounds well suited to the development of new antibiotic drugs.

Present findings suggest a potential antimicrobial activity of white tea against gram positive bacteria; however, complementary studies should be conducted to further evaluate this biological property.

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