

Antibacterial Activity Of The Crude Extract Of Chinese Green Tea (*Camellia Sinensis*) On *Listeria Monocytogenes*

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Abstract

The antibacterial activity of the methanol and aqueous extract of *Camellia sinensis* on *Listeria monocytogenes* were investigated using Agar-gel diffusion, paper disk diffusion and microbroth dilution techniques. The results obtained showed that methanol and water extract exhibited antibacterial activities against *Listeria monocytogenes*. The leave extract produced inhibition zone ranging from 10.0 – 20.1mm against the test bacteria. The methanol extracts of the test plant produces larger zones of inhibition against the bacteria than the water extract. The minimum inhibitory concentration (MIC) for the methanol and water leave extract was 0.26mg/ml and 0.68mg/ml respectively.

INTRODUCTION

Green tea is a non-fermented tea. The tea is an infusion of flavorful leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols [1,2]. Toda et al [3] also showed that moderate daily consumption of green tea killed *Staphylococcus aureus* and other harmful bacteria.

Recent reports however indicate the tea's antibacterial and bactericidal properties on various bacterial strains isolated from patients with infected root canal [4]. Subsequently, several studies on the antimicrobial properties of Japanese tea have been reported [3,5,6]. The antibacterial activity of Turkish tea against *Campylobacter* sp and the protective activity of tea against infection by *Vibrio cholera* 01 have also been reported [7,8].

Listeria monocytogenes a gram-positive bacterium, that is salt resistant and highly adapted. This organism is motile, psychrophil, and occurs everywhere in the environment. It is isolated from silo, vegetable, dairy foods, red meat, ready-to-eat food products etc. it causes listeriosis in human and other animal and birds. The organism is recognized as a food-borne pathogen.

Prompted by these report, the study is therefore aimed at investigating the antibacterial activity of Chinese green tea on *Listeria monocytogenes*.

MATERIALS AND METHODS

PLANT COLLECTION

The air-dried leaves of Chinese green tea (*Camellia sinensis*) were collected from Zhejiang provincial Department of Agriculture, Hangzhou, China. The leaves were cut into pieces and grinded into powdery form using a sterile electric grinder. The soluble ingredients in the grounded plant part was then extracted by solubilization using ethanol and water as different solvents.

AQUEOUS EXTRACTION

The aqueous extractions of the water-soluble ingredients were carried out using the method as described by Asuzu [9]. 15g of each of the grounded leaves were extracted by successive soaking for 2 days using 35ml of distilled water in a 250ml sterile conical flask. The extracts were filtered using Whatman filter paper No 1. The filtrates were concentrated in vacuum at 60°C and stored in universal bottles and refrigerated at 4°C prior to use.

METHANOL EXTRACTION

The methanol extractions of the active ingredient of the leaves were carried out using the method as described by Harbone [10]. 25g of the grinded leaves were Soxhlet extracted using 250ml of 95% methanol. The extraction lasted for six hours. The volatile oil obtained was concentrated by evaporation using water bath at 100°C.

TEST ORGANISM

The strain used in this work was *Listeria monocytogenes* type 4a (food origin) obtained from culture collection centre at Hebrew University Israel. The bacteria was maintained by weekly transfer in a chemically defined medium and tryptic soy broth (TSB) and distributed in 5ml volumes in screw-capped tubes. Cells were grown at 37°C for 48h and cultures were kept at 4°C.

ANTIBACTERIAL SUSCEPTIBILITY TESTING

The antibacterial tests of the leave extracts were tested on the test bacteria using the agar-gel diffusion inhibition test and paper disk diffusion inhibition test. In the agar-gel diffusion inhibition test as described by Opara and Anasa [11], 0.2ml of a 24h broth culture (106cfu/ml) of the bacteria was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller-Hinton agar plates. Three wells of about 6.0mm diameter were aseptically punched on agar-plate using a sterile cork borer allowing at least 30mm between adjacent wells and between peripheral wells and the edge of the petri dish. Fixed volumes (0.1ml) of the leave extract were then introduced into the wells in the plates. A control well was in the centre with 0.01ml of the extracting solvent. The plates incubated at 37°C for 24h for the test bacteria. The plates were duplicated in all the experiments.

In the paper disc diffusion test, sterile paper discs were soaked in the leave extract for 2h. 0.2ml of a 24h broth culture (106cfu/ml) of the bacteria specie was spread on the surface of gelled sterile Mueller-Hinton agar plates. The paper discs containing the extracts were placed at different areas on the surface of each plate. The plates were incubated at 37°C for 24h. Antibacterial activity of the extract against the test bacteria was indicated by growth-free “zone of inhibition” near the respective disc.

MINIMUM INHIBITORY CONCENTRATION

The agar diffusion method described by Ver-poorte [12] was used. The extracts were incorporated into Mueller-Hinton broth at concentration ranging from 0.01-10mg/ml. A control tube containing the growth medium and the bacteria was set-up. The mixtures were incubated at appropriate temperature of 37°C for 24h. The minimum inhibitory concentration (MIC) of the extracts were regarded as the lowest concentration of the extract that did not permit and turbidity or growth of the test organism.

RESULTS

The methanol extract of the leave of *Camellia sinensis* showed various levels of antibacterial activity when tested by both methods. Whereas the aqueous extract showed antibacterial activity only when tested by the paper disc diffusion method (Table 1 –2). The methanolic extract of the leave of *Camellia sinensis* possess greater antibacterial properties against *Listeria monocytogenes* as against the water extract.

Table 2 showed the antibacterial susceptibility of the aqueous extract of the leave of *Camellia sinensis* on *L. monocytogenes*. There was no antibacterial or antilisteric activity of the aqueous leave extract on the test organism using Agar-gel diffusion. Higher diameter zones of inhibition was obtained with the paper disc method than with Agar-gel diffusion method on the test organism and for both extraction methods. The minimum inhibitory concentration (MIC) of the methanol extract 0.26mg/ml were lower than those of the extract of 0.68mg/ml as can be seen in table 3. This indicate that the extracts is very active and possess antilisteric properties.

Figure 1

Table 1: Antibacterial activity of the methanol extract of leave of *Camellia sinensis* on *L. monocytogenes*.

Mean zone of inhibition of organism (mm)		
Microorganism	Paper disc diffusion	Agar-gel diffusion
<i>L. monocytogenes</i>	20.1	15.0

Figure 2

Table 2: Antibacterial activity of the Aqueous extract of leave of *Camellia sinensis* on *L. monocytogenes*

Mean zone of inhibition of organism (mm)	
Microorganism	Paper disc diffusion
<i>L. monocytogenes</i>	10.0

Figure 3

Table 3: Minimum inhibitory concentration of the leave extract of *Camellia sinensis* on *L. monocytogenes*.

Microorganism	Methanol extract 10mg/ml	Water extract 10mg/ml
<i>L. monocytogenes</i>	0.26	0.68

DISCUSSION

The result of the study showed that the leave extract of *Camellia sinensis* produced zones of inhibition against *Listeria monocytogenes*. This indicates the presence of potent antibacterial activity, which confirms its use as anti-

infective.

Although both the methanol and water extract of the leave of *Camellia sinensis* produced inhibitory actions against.

Listeria monocytogenes, methanol extracts showed more inhibitory effects than the water extract. This tends to show that the active ingredients in the leaves were better extracted with methanol than water. Akunyili et al [13] observed a similar result when they worked with stem bark of *Kigelia pinnata*.

Toda et al [14] reported that daily consumption of green tea can kill gram positive *Staphylococcus aureus* and other harmful bacteria. Also it have been reported [15,16,17,18,19] that the green tea contain catechin and polyphenols. These compounds have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties. This suggests that these compounds could be responsible for the inhibitory of *L.monocytogenes* used in this study.

The two methods used to test the antibacterial activity of the leave extract proved to be good, but the paper disc diffusion method tend to show wider zones of inhibition than the agar-gel diffusion methods.

From the result obtained in Table 3, it showed that at low doses of 0.26mg/ml and 0.68mg/ml the crude extract of the methanol or probably the water extract would inhibit the effect of the aetiologic agent causing these diseases (listeriosis). This gives credence to its ethnopharmacological use as a remedy to treat infections and diseases caused by the organism.

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