African Journal of Biotechnology Vol. 7 (10), pp. 1571-1573, 16 May, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB06.316
ISSN 1684–5315 © 2008 Academic Journals

Short Communication

Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*

Mbata, T. I.[1]*, Debiao, L. U.[2] and Saikia, A.[3]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P. M. B 5025, Awka, Nigeria.
[2]Cash Crops Bureau, Zhejianq Provincial Department of Agriculture, Hangzhou, 310020, China.
[3]Department of Horticulture, Assam Agricultural University, Jorhat 785013, Assam, India.

Accepted 6 October, 2006

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 L. *monocytogenes*. The leaf extract produced inhibition zone ranging from 10.0 – 20.1 mm 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 leaf extract was 0.26 and 0.68 mg/ml, respectively.

Key words: Antibacterial activity, Chinese green tea, *Listeria monocytogenes*.

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 (Sofowara, 1984; Opara, 1992). Toda et al. (1989a) 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 (Horiba, 1991). Subsequently, several studies on the antimicrobial properties of Japanese tea have been reported (Toda et al., 1989a; Sakanaka, et al., 1989; Okubo et al., 1989). 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 (Diker, 1991; Toda, 1991).

Listeria monocytogenes is 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, other animals and birds. The organism is recognized as a food-borne pathogen. The study is aimed at investigating the antibacterial activity of Chinese green tea on *L. monocytogenes*.

MATERIALS AND METHODS

Plant collection

The air-dried leaves of Chinese green tea (*Camellia sinensis*) were collected from Zhejianq 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 were then extracted by solubilization using ethanol and water as different solvents.

*Corresponding author. E-mail: theoiyke@yahoo.com.

Table 1. Antibacterial activity of the methanol and aqueous extracts of leave of *C. sinensis* on *L. monocytogenes*.

Extract	Mean zone of inhibition of organism (mm)		MIC*
	Paper disc diffusion	Agar-gel diffusion	
Methanol	20.1	15.0	0.26
Water	10.0	-	0.68

*Concentration of extract used is 10 mg/ml.

Aqueous extraction

The aqueous extractions of the water-soluble ingredients were carried out using the method as described by Asuzu (1986). 15 g of each of the grounded leaves were extracted by successive soaking for 2 days using 35 ml of distilled water in a 250 ml 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 (1994). 25 g of the grinded leaves were soxhlet extracted using 250 ml 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 *L. 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 5 ml volumes in screw-capped tubes. Cells were grown at 37°C for 48 h and cultures were kept at 4°C.

Antibacterial susceptibility testing

The antibacterial tests of the leaf 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 (1993), 0.2 ml of a 24 h broth culture (10^6 cfu/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 6.0 mm diameter were aseptically punched on agar-plate using a sterile cork borer allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the petri dish. Fixed volumes (0.1 ml) of the leaf extract were then introduced into the wells in the plates. A control well was in the centre with 0.01 ml of the extracting solvent. The plates were incubated at 37°C for 24 h 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 leaf extract for 2 h. 0.2 ml of a 24 h broth culture (10^6 cfu/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 24 h. 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 et al. (1982) was used. The extracts were incorporated into Mueller-Hinton broth at concentration ranging from 0.01-10 mg/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 24 h. The minimum inhibitory concentration (MIC) of the extracts was regarded as the lowest concentration of the extract that did not permit turbidity or growth of the test organism.

RESULTS

The methanol extract of the leaf of *C. 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). The methanolic extract of the leaves of *C. sinensis* possess greater antibacterial properties against *L. monocytogenes* compared to the water extract. The table showed the antibacterial susceptibility of the aqueous extract of the leaves of *C. sinensis* on *L. monocytogenes*. There was no antibacterial or antilisteric activity of the aqueous leaf extract on the test organism using agar-gel diffusion. Higher diameter zones of inhibition were 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 0.26 mg/ml were lower than those of the extract of 0.68 mg/ml as can be seen in Table 1. This indicates that the extract is very active and possesses antilisteric properties.

DISCUSSION

The result of the study showed that the leaf extract of *C.sinensis* produced zones of inhibition against *L. 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 leaves of *C. sinensis* produced inhibitory actions against *L. 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. (1991) observed a similar result when they worked with stem bark of *Kigelia pinnata*.

Toda et al. (1989b) reported that daily consumption of green tea can kill gram positive *S. aureus* and other harmful bacteria. Also it has been reported (Ahn et al., 1991; Wakayama et al., 1993; Makhtar et al., 1994; Sakanaka et al., 1996; Kuroda and Hara, 1999) that the green tea contains 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 inhibition of *L. monocytogenes* used in this study.

The two methods used to test the antibacterial activity of the leaf 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 the table, it showed that at low doses of 0.26 and 0.68 mg/ml of 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.

REFERENCES

Ahn Y-J, Kawamura T, Kim M, Yamamoto T, Mitsuoka T (1991). Tea Polyphenols: Selective growth inhibitors of *Clostridium* spp. Agric Biol Chem. 55: 1425-1426.

Akunyili AN, Houghton DJ, Romana (1991). Antibacterial activity of the stem bark of *Kigelia pinnata*. J. Ethanopharmacol, 2: 173 – 177.

Asuzu IU (1986). Pharmacological evaluation of folkore of Sphenostylic Slenocarpa. J. Ethanopharmacol, 16: 236-267.

Diker KS, Akan M, Hascelik G, Yurdakok M (1991). The bactericidal activity of tea against *Campylobacter jejuni* and *Campylobacter coli*. Letters Appl. Microbiol., 12: 34-35.

Harbone NV (1994). Phytochemical method. A guide to modern techniques of plant analysis 2nd ed, Chapman and Hall London. p. 425.

Horiba N, Maekawa Y, Ito M, Yamamoto T (1991). A pilot study of japanese green tea as a medicament, antibacterial and bactericidal effects. J. Endodonitics 17: 122-124.

Kuroda Y, Hara Y (1999). Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat. Res. 436(1): 60-67.

Makhtar H, Hatiyar S, Agarwal P (1994). Green tea and skin anticarcinogenic effect. J. Invest Dermatol. 102: 3-7.

Okubo S, Ikigia H, Toda M, Shimamura T (1989). The anti-haemolysin activity of tea and coffee. Lett. Appl. Microbiol. 9: 65-66.

Opara AA (1992). The Kargasok tea. Medicare J. 5(3): 3-5.

Opara AA, Ansa MA (1993). The antibacterial activity of tea and coffee on selected organisms. J. Med. Lab. Sci. 3: 45-48.

Sakanaka S, Aizawa M, Kim M, Yamamoto T (1996). Inhibitory effect of green tea polyphenols on growth and cellular adherence of an oral bacterium, Porphyromonas gingivalis. Biosci. Biotechnol Biochem 60: 745-749.

Sakanaka S, Kim M, Taniguchi M, Yamamoto T (1989). Antibacterial substances in Japanese green tea extract against *Streptococcus mutans* a cariogenic bacterium. Agric Biol. Chem. 53: 2307-2311.

Sofowara A (1984). A medicinal plants and Traditional medicine in Africa, John Wiley Chichester, p. 256.

Toda M, Okubo S, Hiyoshi R, Shimamura T (1989a). Antibacterial and bactericidal activities of Japanese green tea. Jpn. J. Bacteriol. 44(4): 669-672.

Toda M, Okubo S, Ikigai H, Suzuki T Shimamura T (1991). The protective activity of the tea against infection by *Vibrio cholerae* 01. J. Appl Bacterial. 70: 109 – 112.

Toda M, Okubo S, Hiyoshi R, Shimamura T (1989b). Antibacterial and bacteriacidal activities of Japanese green tea. Nippon Saikingaku Zasshi 44(4): 669-672.

Ver-porte R, Ijin A, Tsoui A, Vandourne H, Baerheim. M (1982). Medicinal plants of Surinam. Antimicrobial activity of medicinal plant J. Ethanopharmacol. 5: 221-226.

Wakayama M, Suzuki K, Toda M, Okubo S, Hara Y, Shimamura T (1993). Inhibition of the infectivity of influenza virus by tea polyphenols. Antiviral Res. 1: 289-299.