Clinical Research

Antibacterial Activity of Cinnamomum Zeylanicum Bark Against Escherichia Coli Isolates

Cinnamomum Zeylanicum Kabuğunun Escherichia Coli Izolatlarına Karşı Antibakteriyel Aktivitesi

Birgül Kaçmaz [1], İlker Durak [2]

1. Kırıkkale University, Faculty of Medicine, Dept. of Infectious Diseases and Clinical Microbiology, Kirikkale, Turkey
2. Ankara University, Faculty of Medicine, Department of Biochemistry, Sihhiye, Ankara, Turkey

ABSTRACT

Objectives: *The aim of this study was to investigate the antibacterial activity of Cinnamomum zeylanicum bark (Czb) water extract against Escherichia coli isolates. The synergistic activity of Czb water extract with various antimicrobial drugs against Escherichia coli isolates (extended spectrum beta lactamase-ESBL- producing and non-ESBL producing) was also investigated.*

Materials and Methods: *Isolates were isolated from various clinical specimens. Antibacterial activity of plant extract was determined by agar dilution method. The synergistic effect of Czb extract with certain antibiotics (ciprofloxacin, ofloxacin, ceftriaxone, amoxicillin-clavulonate, imipenem, amikacin, trimethoprim-sulfamethoxazole, piperacillin-tazobactam) was assayed by the disc diffusion method on agar containing subinhibitory concentrations of the extract.*

Results: *The extract showed antibacterial activity against all tested E. coli isolates. In non-ESBL producing isolates, the extract showed statistically significant synergism with all of the selected antibiotics. In ESBL producing isolates, significant synergism was detected only with imipenem.*

Conclusions: *In this study, in vitro Czb water extract was found to have antibacterial activity against E. coli isolates. Also, the extract was potentiated the antibacterial effects of the selected antibiotics. We thought that extract used in the study can contribute to the development of new antibacterial agents in the anti-E.coli isolates treatment regimen.*

Keywords: *antibacterial activity; Cinnamomum zeylanicum; medicinal plants*

ÖZET

Amaç: *Bu çalışmanın amacı Cinnamomum zeylanicum kabuğunun (Czb) su ekstrat formunun Escherichia coli izolatlarına karşı antibakteriyel etkinliğinin araştırılmasıdır. Aynı zamanda ekstratın çeşitli antimikrobiyal ajanlar ile genişlemiş spektrumlu beta laktamaz (GSBL) üreten ve GSBL üretmeyen E. coli izolatlarına karşı sinerjistik etkisi de araştırılmıştır.*

Materyal ve Metod: *İzolatlar çeşitli klinik örneklerden elde edilmiştir. Ekstratın antibakteriyel etkinliği agar dilüsyon metodla saptanmıştır. Ekstratın bazı antibiyotiklere (siprofloksasin, ofloksasin, seftriakson, amoksisilin-klavulonat, imipenem, amikasin, trimetoprim-sulfametoksazol, piperasilin-tazobaktam) karşı sinerjistik etkisi ekstratın subinhibitör konsantrasyonunu içeren agarda disk difüzyon yöntemi ile değerlendirilmiştir.*

Bulgular: *Ekstrat çalışmada kullanılan E. coli izolatlarına karşı antibakteriyel etki göstermiştir. GSBL üretmeyen izolatlarda, ekstratın tüm antibiyotikler ile sinerjistik etki gösterdiği saptanmıştır. GSBL üreten izolatlarda ise, sinerjistik etki sadece imipenemde tespit edilmiştir.*

Sonuçlar: *Bu çalışmada, in vitro Czb ekstratının E. coli izolatlarına karşı antibakteriyel etkisi olduğu bulunmuştur. Aynı zamanda ekstratın seçilmiş olan antibiyotiklerin antibakteriyel etkisini arttırdığı da saptanmıştır. Çalışmada kullanılan extratın E. coli izolatlarına karşı yeni antibakteriyal ajanların gelişimine katkısı olabileceğini düşünüyoruz.*

Anahtar Kelimeler: *antibakteriyel etki; Cinnamomum zeylanicum; şifalı bitkiler*

Contact Information
Corresponding Author: Dr. Birgül Kaçmaz
Address: Kırıkkale Üniversitesi Tıp Fakültesi, Enfeksiyon Hast. ve Kl. Mikrob. AD. Yahşihan, Kırıkkale, Turkey
Phone : +90 (318) 333 50 00 / 5067 **Gsm:** +90 (532) 743 72 29
E-mail: kacmazbirgul@mynet.com
Submitted: 19.11.2015
Accepted: 08.12.2015

Bu çalışma; 9-12 Mayıs 2012 tarihinde Green Park Hotel Pendik, İstanbul'da düzenlenen Türkiye EKMUD Kongresinde, yazılı poster olarak sunulmuştur.

INTRODUCTION

Various products derived from plants have been used for treatment of a wide variety of diseases by humans for a long period of time. According to a report by World Health Organization (WHO), traditional medicine is based on drugs derived from plants (1). Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (2).

Cinnamomum zeylanicum bark (Czb) is an aromatic plant that is used as a spice all over the world. The major constituent of Czb is the volatile oil which contains cinnamic aldehyde, eugenol, terpenes and tannins and mucilage. These substances provide antimicrobial activity alone or in combinations (3).

The reports from China have shown that inner bark, oil and fruit parts of cinnamomum zeylanicum have good antimicrobial activity. Water, ethanol and acetone extracts of the plant were tested against urinary tract pathogenic bacteria. Ethanolic extract was found to be more active than acetone and water extract (4). Ethanol has a stronger extraction capacity and thus the number of active constituents in ethanolic extract is greater than acetone and water extract. It is probable that the active compounds present in water extracts are different from those responsible for the action in other type of extracts. However, the water extracts of some plants indicated a higher antimicrobial activity than their ethanolic counterparts. Each type of extract contains multiple and different antimicrobial agents which act in different ways on various bacterial isolates (5).

Novel antibacterial actions of plant extracts or phytocompounds have been documented which include inhibition of MDR-efflux pump, β-lactamase activity and anti-antibiotic resistance properties (6-8).

It was reported that natural products in cinnamomum zeylanicum show antibacterial effect on E.coli isolates by inhibiting the carboxyltransferase component of E. coli acetyl-CoA carboxylase (9).

In this study, antibacterial activity of Czb water extract against Escherichia coli isolates was investigated. In vitro synergistic action of Czb water extract with some antibacterial drugs used against E. coli isolates was also evaluated by using the disc diffusion method.

MATERIALS AND METHODS

Plant samples: In the assays, Czb obtained from retail was used.

Preparation of plant extracts: Extracts were prepared by soaking and mixturing the Czb powder (5 % w/v) in distilled water for 36 hours. Upper part was removed after centrifugation at 5000 rpm for 15 min and used in the assays.

Bacterial isolates: A total of 21 microorganisms composed of one reference isolate (E. coli ATCC 25922) and 20 clinical isolates (10 extended spectrum beta lactamase (ESBL) producing and 10 non-ESBL producing E. coli) were used in the study. Clinical isolates were isolated from various (urine, and wound) clinical specimens.

Detection of ESBL production: Confirmatory testing for ESBL presence was performed by disk diffusion using ceftazidime and cefotaxime disks, with and without clavulanic acid. Isolates demonstrating a clavulonic acid effect, defined as an increase in zone diameter of ≥5 mm in the presence of clavulonic acid for at least one antibiotics, were considered to be ESBL producers.10 Klebsiella pneumoniae ATCC 700603 (ESBL positive) and E. coli ATCC 25922 (ESBL negative) were used as quality control for ESBL detection. Antibiotic potency of the cefotaxime and ceftazidime disks was standardized against the reference isolate, E. coli ATCC 25922. Antibiotic disks were obtained from Oxoid Ltd, Basingstoke, UK.

Antimicrobial activity: Antimicrobial activity of plant extract was determined by agar dilution method (10). Test plates were prepared with 19 ml of Mueller-Hinton agar (MHA) (Oxoid, Unipath LTD., Basingstoke, Hampshire, England), and 1 ml of 2-fold dilutions of plant extract (mg/ml). After cooling and drying, the plates were inoculated with 10 µl of the 106 cfu/ml suspension. Plates were incubated for 48 h at 35°C, and the minimal inhibitory concentration (MIC) were defined as the lowest concentration of plant extract that inhibits visible growth of microorganisms spots, and the MIC 90% was calculated. Tests were repeated three times.

Synergy assay: One-fourth the MIC 90% was thought as the sub-inhibitory concentration of the plant extract in the synergism assays (11). Eigth drugs were evaluated: ciprofloxacin (5 µg), ofloxacin (5 µg), ceftriaxone (30 µg), amoxicillin-clavulonate (20/10 µg), imipenem (10

µg), amikacin (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), piperacillin-tazobactam(100/10 µg). Antibiotic potency of the disks was standardized against the reference isolate, E. coli ATCC 25922. Antibiotic disks were obtained from Oxoid Ltd, Basingstoke, UK. Two antibiogram sets were performed in duplicate for each E. coli isolate in control plates, with plain MHA, and in plates containing MHA plus one-fourth the MIC 90% of the plant extract. The diameters (mm) of the each inhibitory zone were recorded after incubation for 24-48h at 35°C. The assays were performed in triplicates and the inhibition zone diameter (IZD) was measured separately and the average of the three replicates were recorded.

Statistical analysis: Results from the synergism assays were subjected to the Wilcoxon nonparametric test to compare the values (mm) of the inhibitory zones obtained by the disk diffusion method (Minitab Statistical Software version 13.32). P values <0,05 were considered as significant.

RESULTS

MIC values of the extract were determined as an evaluation of antibacterial activity against the bacteria tested. MIC values were found to be 2.5 mg/ml and sub inhibitor concentrations 0.625 mg/ml for 21 E. coli isolates.

The results of the in vitro interaction studies of the extract and the selected antibiotics against bacteria were presented in Table I and II.

Table I: The combination of the extract and selected antibiotics against non-ESBL producing E.coli (10 clinical isolates and one reference isolate).

Antibiotics	Antibiotic alone (mm)*	Antibiotic plus plant extract (mm)*	% change in IZD**	p values
Ciprofloxacin	28,50	35,82	26	,018***
Ofloxacin	26,45	32,45	23	,020***
Ceftriaxone	31,36	38,00	21	,008***
Amoxicillin-clavulonate	26,36	32,09	22	,008***
Amikacin	21,45	28,64	34	,015***
Trimethoprim-Sulfamethoxazole	23,82	29,09	22	,018***
Imipenem	35,91	41,82	16	,003***
Piperacillin-tazobactam	32,00	38,73	21	,005***

** The values of all isolates are average figures of three replicates.*
*** IZD: inhibition zone diameter.*
**** p values <0,05 were considered as significant.*

Table II: The combination of the plant extract and selected antibiotics against ESBL producing E.coli (10 clinical isolates).

Antibiotics	Antibiotic alone (mm)*	Antibiotic plus plant extract (mm)*	% change in IZD**	p values
Ciprofloxacin	11,40	14,70	29	,249
Ofloxacin	10,00	9,00	-10	,705
Ceftriaxone	10,00	9,00	-10	,705
Amoxicillin-clavulonate	14,10	16,90	20	,066
Amikacin	18,70	19,40	4	,723
Trimethoprim-Sulfamethoxazole	9,60	8,20	-15	,715
Imipenem	34,90	40,60	16	,007***
Piperacillin-tazobactam	22,40	21,90	-2	,674

** The values of all isolates are average figures of three replicates.*
*** IZD: inhibition zone diameter.*
**** p values <0,05 were considered as significant.*

Synergy was observed between all tested antibiotics and plant extract in non- ESBL producing E. coli (10 clinical isolates and one reference isolate).

The increasing IZDs were detected against ciprofloxacin, amoxicillin-clavulonate, amikacin and imipenem at different rates in ESBL producing E. coli. Based on statistical analyses, the increase in IZD was significant only against imipenem (p: 0,007 p<0,05). No sinergism was established between the tested antibiotics and the extract in ESBL producing E. coli, except imipenem.

DISCUSSION

In the present study, the antibacterial activity of Czb water extract was detected on E. coli isolates (both ESBL-producing and non ESBL-producing).

Plant extracts are commonly prepared with water, as infusions and decoctions, in traditional medicine (12). We also preferred to use water extract of the plant in this study.

Few plants' extracts and phytocompounds exhibited synergistic interaction with antibiotics against Gram-positive bacteria. Interaction of plant extract with classical antibiotics may also be due to different mechanisms (13, 14). In our study, Czb water extract showed significant synergistic interaction with ciprofloxacin, ofloxacin, ceftriaxone, amoxicillin-clavulonate, imipenem, amikacin,

trimethoprim-sulfamethoxazole, and piperacillin-tazobactam against non ESBL-producing E.coli isolates.

An increase in IZDs of ciprofloxacin, amoxicillin-clavulonate, amikacin and imipenem was also detected in different ratios for ESBL producing isolates. The increase in IZD (synergistic activity) was found to be statistically significant for only imipenem.

Such plants or active fractions may enhance the activity of classical antibiotics by above-mentioned or yet unknown mode of actions. Various plant extracts have been reported to have synergistic interactions with antibiotics (13, 14).

According to the data obtained with ESBL producing isolates in this study, ESBL enzymes were found to inhibit or decrease the antibacterial synergism between Czb extract and the antibiotics, excluding imipenem. Currently, we could not be able to explain the mechanism of the above-mentioned effect of the ESBL enzymes. Further studies need to be carried out in order to establish the mechanism of such interaction.

In conclusion, Czb water extract was also found to have antibacterial effect on E.coli isolates. We suggest that, consumption of Czb water extract may be beneficial in treatment of infections caused by E. coli. Moreover, due to synergistic action with antibiotics, Czb water extract could help treatment of infections more effectively when used with antibiotics. We thought that extract used in the study can contribute to the development of new antibacterial agents in the anti-E.coli isolates treatment regimen.

REFERENCES

1. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine 2006; 6:39-46.

2. Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol 2007;117:112-9.

3. Kamath JV, Rana AC, Chowdhury AR. Pro-healing effect of Cinnamomum zeylanicum bark. Phytother Res 2003;17:970-2.

4. Sharma A, Chandraker S, Patel VK, Ramteke P. Antibacterial Activity of Medicinal Plants Against Pathogens causing Complicated Urinary Tract Infections. Indian J Pharm Sci 2009; 71:136-9.

5. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts. J Nat Med 2008;62:259-62.

6. Stermitz FR, Lorenz P, Tawara JN, Zenewicz A, Lewis K. Synergy in a medicinal plants: antimicrobial action of berberine potentiated by 5'- methoxyhydnocarpin, a multi drug pump inhibitior. Proc Natl Acad Sci 2000;97:1433-7.

7. Yam TS, Hamilton-Miller JMT, Shah S. The effect of a component of tea (Camellia sinensis) on methicillin resistance, PBP2' synthesis, and b-lactamase production in S. aureus. J Antimicrobial Chemother 1998;42:211-6.

8. Lee CK, Kin H, Moon KH, Shun KH. Screening and isolation of antibiotic resistance inhibitors from herb materials-resistance inhibition of volatile components of Korean aromatic herbs. Arch Pharm Res 1998;21:62-6.

9. Meades Jr G, Henken RL, Waldrop GL, Rahman M, Gilman SD, Kamatou GP, et al. Constituents of cinnamon inhibit bacterial acetyl coA carboxylase. Planta Med 2010;76:1570-5.

10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 2009; nineteenth informational supplement M100-S19 Vol. 29 No. 3.

11. Mahon CR, Manuselis Jr G. Textbook of diagnostic microbiology, WB, Saunders, Philadelphia, 1995: 1134-8.

12. Gonçalves FA, Andrade Neto M, Bezerra JNS, Macrae A, Sousa OV, Fonteles-Fılho AA, et al. Antibacterial activity of guava, psidium guajava linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from seabob shrimp, xiphopenaeus kroyeri (heller). Rev Inst Med Trop S Paulo 2008;50: 11-5.

13. Zhao W, Hu Z, Okubo S, Hara Y, Shimamura T. Mechanism of synergy between epigallocatechin gallate and beta-lactams against methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2001;45: 1737-42.

14. Aqil F, Khan MSA, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant Staphylococcus aureus. J Basic Microbiol 2005;45: 106-14.