

ORIGINAL ARTICLE

ANTIBACTERIAL ACTIVITIES OF *EMBLICA OFFICINALIS* AND *CORIANDRUM SATIVUM* AGAINST GRAM NEGATIVE URINARY PATHOGENS

SABAHAT SAEED AND PERWEEN TARIQ

Department of Microbiology, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

Present investigation is focused on antibacterial potential of aqueous infusions and aqueous decoctions of *Emblica officinalis* (amla) and *Coriandrum sativum* (coriander) against 345 bacterial isolates belonging to 6 different genera of Gram negative bacterial population isolated from urine specimens by employing well diffusion technique. Aqueous infusion and decoction of *Emblica officinalis* exhibited potent antibacterial activity against *Escherichia coli* (270), *Klebsiella pneumoniae* (51), *K. ozaenae* (3), *Proteus mirabilis* (5), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (1), *S. paratyphi* A (2), *S. paratyphi* B (1) and *Serratia marcescens* (2) but did not show any antibacterial activity against Gram negative urinary pathogens.

Keywords: *Emblica officinalis*, *Coriandrum sativum*, well diffusion technique, antibacterial activity, Gram negative bacteria.

INTRODUCTION

Urinary tract infections are the most commonly observed infections in clinical practice they also contribute the most common nosocomial infection in many hospitals, and accounts for approximately 35% of all hospital acquired infections (Hvidberg *et al.*, 2000). The enterobacteriaceae, were the most common pathogen detected, causing 84.3% of the urinary tract infections (Gales *et al.*, 2000). *Escherichia coli* causes about 85% of community acquired urinary tract infections and more than 80% of cases of uncomplicated pyelonephritis. The medically equally important *Klebsiella* accounts for 6-17% of all nosocomial urinary tract infections. *Proteus* is also a common cause of urinary tract infection in individuals with complicated urinary tract infections (Noor *et al.*, 2004).

Multiple microbial resistance among Gram-negative organisms has been a long term and well-recognized problem with urinary tract infections. Resistance has been observed in multiple genera including *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia* and *Pseudomonas* (Noor *et al.*, 2004). Recently there has been a renewed interest in improving health and fitness through the use of natural products. Herbs and spices are the most important part of human diet. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value (DeSouza, 2005), which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices (Chaudhry and Tariq, 2006).

Emblica officinalis is member of small genus *Emblica*. The fruit is commonly known as amla and is highly valued in traditional Indian medicine (Scartezzini *et al.*, 2006). In Unani medicine the dried fruits of amla are used to treat hemorrhage, diarrhea and dysentery (Parrotta, 2001). In addition, the fruit of *E. officinalis* is diuretic (Anonymous, 2006), adaptogenic (Rage *et al.*, 1999), hepatoprotective (Jeena *et al.*, 1999; Jose and Kuttan, 2000), antitumor (Jose *et al.*, 2001), hypocholesterolemic (Kim *et al.*, 2005), antioxidant (Bhattacharya *et al.*, 1999) and antiulcerogenic (Sairam *et al.*, 2002). In previous studies the fruits are also reported to be anti-inflammatory (Sharma *et al.*, 2003), analgesic and antipyretic. Several constituents of *E. officinalis* fruit has been identified, mainly the hydrolysable tannins, emblicanin A, emblicanin B, punigluconin and pedunculagin (Perianayagam *et al.*, 2005). Emblicanin A and B have been proposed to be the active constituents with significant *in vitro* antioxidant activity (Ghosal *et al.*, 1996).

Earlier studies have demonstrated potent antimicrobial properties of *E. officinalis* (Ahmed *et al.*, 1998) and it is used as antiviral for cold and flu. In the respiratory infections, it has an antibiotic activity against a wide range of bacteria, used traditionally in the treatment of lungs (Chopra and Simon, 2000). It also has been antifungal activity *in vitro* (Dutta *et al.*, 1998).

Coriandrum sativum (coriander) is considered both as an herb and a spice. Both its leaves and seeds are used as seasoning condiment. Coriander seeds have health-supporting reputation that is high on the list of healing spices. It has traditionally been referred to as antidiabetic (Gray and Flatt, 1999), anti-inflammatory and cholesterol

Corresponding author: sabahatsaeed2003@yahoo.com

lowering (Chithra and Leelamma, 1997). In addition, it is also used as carminative, diuretic, stimulant, stomachic, refrigerent, aphrodisiac, analgesic (Chaudhry and Tariq, 2006) and hypoglycemic (Waheed *et al.*, 2006).

The seeds of *C. sativum* contain 0.5-1 % essential oil and are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol, and linalool. Coriander's flavonoides include quercetin, kaempferol, rhamnetin and epigenin. It also contains active phenolic acid compounds including caffeic and chlorogenic acid. Research also suggests that the volatile oils founds in the leaves of *C. sativum* plant may have antimicrobial properties against food borne pathogens, such as *Salmonella* species (Kubo *et al.*, 2004).

In view of the above the present study was conducted to evaluate the antibacterial potential of aqueous infusions and decoctions of *E. officinalis* and *C. sativum* against 345 different isolates belonging to 6 genera of gram negative bacilli isolated from urine specimens, viz., *Escherichia coli* (270), *Klebsiella pneumoniae* (51), *K. ozaenae* (3), *Proteus mirabilis* (5), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (1), *S. paratyphi* A (2), *S. paratyphi* B (1) and *Serratia marcescens* (2).

MATERIALS AND METHODS

Maintenance of isolates

A total of 345 isolates belonging to 6 different species of Gram negative bacilli isolated from urine specimens were maintained on tryptone soy agar (TSA) (Oxoid).

Preparation of aqueous infusions

Aqueous infusions of *E. officinalis* and *C. sativum* were prepared by steeping 20g in 100 ml sterile distilled water in separate sterile flasks. The flasks were kept for two days with occasional shaking and the contents were filtered.

Preparation of aqueous decoctions

Aqueous decoctions of *E. officinalis* and *C. sativum* were prepared by boiling 20g in 100 ml sterile distilled water for 15 minutes. The flasks were then plugged and removed from heat and allowed to cool and then filtered.

SCREENING OF ANTIBACTERIAL ACTIVITY

Media

Mueller-Hinton agar (MHA) (Merck) was used as base medium for screening of antibacterial activity and Mueller-Hinton broth (MHB) (Merck) for preparation of inoculum.

Preparation of McFarland Nephelometer standard

McFarland tube number 0.5 was prepared by mixing 9.95 ml 1% sulphuric acid in MHB and 0.05 ml 1% barium

chloride in distilled water in order to estimate bacterial density (Saeed and Tariq, 2006). The tube was sealed and used for comparison of bacterial suspension with standard whenever required.

Preparation and standardization of inoculum

Four to five colonies from pure growth of each test organism were transferred to 5 ml of MHB. The broth was incubated at 35-37°C for 18-24 hours. The turbidity of the culture was compared to 0.5 McFarland Nephelometer standard to get 150×10^6 CFU/ml, the standardized inoculum suspension was inoculated within 15-20 minutes.

Well diffusion technique

Screening of antibacterial activity was performed by well diffusion technique (Saeed and Tariq, 2005), the MHA plates were seeded with 0.1 ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 100 μ l of each infusion and decoction of *E. officinalis* and *C. sativum* was introduced in the well.

Incubation

The inoculated plates were incubated at 35-37°C for 24 hours and zone of inhibition was measured to the nearest millimeter (mm).

STATISTICAL ANALYSIS

Mean zone of inhibition and standard deviations were calculated.

RESULTS AND DISCUSSION

Three hundred and forty five urinary pathogens belonging to 6 different genera of gram negative bacteria isolated from urine specimens, viz., *Escherichia coli* (270), *Klebsiella pneumoniae* (51), *K. ozaenae* (3), *Proteus mirabilis* (5), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (1), *S. paratyphi* A (2), *S. paratyphi* B (1) and *Serratia marcescens* (2), were used in the present study. The results of *in vitro* antibacterial activity of aqueous infusions and decoctions of *E. officinalis* and *C. sativum* are presented in table 1. The diameter of inhibitory zones recorded includes the size of filter paper discs (6mm in diameter).

Present study indicated great variation in antimicrobial activities of selected spices. The results showed that the aqueous infusion and decoction of *E. officinalis* exhibited maximum activity against *S. typhi* with 20.6 mm and 21.15 mm mean zone of inhibition respectively and also exhibited potent antibacterial activities against all bacterial isolates tested. The minimum activities of

Table 1: Antibacterial activities of aqueous infusion and decoction of *Emblica officinalis* and *Coriandrum sativum*.

S. No.	Organisms	No. of isolates	Mean zone of inhibition \pm Standard deviation			
			<i>Emblica officinalis</i>		<i>Coriandrum sativum</i>	
			Infusion	Decoction	Infusion	Decoction
1	<i>E. coli</i>	270	15.21 \pm 3.19	17.36 \pm 2.23	0	0
2	<i>K. pneumoniae</i>	51	17.39 \pm 3.21	19.30 \pm 2.22	0	0
3	<i>K. ozaenae</i>	3	15.22 \pm 3.58	18.21 \pm 3.82	0	0
4	<i>P. mirabilis</i>	5	12.00 \pm 3.12	15.10 \pm 3.34	0	0
5	<i>P. aeruginosa</i>	10	12.35 \pm 3.22	15.25 \pm 3.12	0	0
6	<i>S. typhi</i>	1	20.60	21.15	0	0
7	<i>S. paratyphi A</i>	2	14.93 \pm 1.15	15.00 \pm 1.32	0	0
8	<i>S. paratyphi B</i>	1	12.50	17.20	0	0
9	<i>S. marcescens</i>	2	10.23 \pm 1.35	12.30 \pm 1.53	0	0

aqueous infusion and decoction were found against *S. marcescens* with 10.23 \pm 1.35 and 12.30 \pm 1.53 respectively in term of mean zone of inhibition \pm standard deviation. Findings of present study are similar to those reported by Khanna and Nag (1973) that constituents of *C. officinalis* have been found to be active against a range of bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Mycobacterium tuberculosis* and *S. typhosa*.

In the present study, the antibacterial activities of aqueous infusion and decoction of *C. sativum* were also evaluated. All tested isolates were found resistant to aqueous infusion and decoction of *C. sativum*. These findings are in fair correlation with the study carried out by Chaudhry and Tariq (2006) who found that decoction of *C. sativum* does not show antibacterial potential against Gram positive and Gram negative bacteria. Similarly, some workers have also found that *C. sativum* does not has antibacterial activity against *S. aureus*, *S. typhi* and *E. coli* (Al-Jedah *et al.*, 2000).

The present study has revealed the importance of natural products to control antibiotic resistant bacteria which are being a threat to human health. This scientific study can serve as an important platform for the development of inexpensive, safe and effective medicines.

REFERENCES

Ahmed J, Mehmood Z and Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.*, **62**: 183-193.

Al-Jedah JH, Ali MZ and Robinson RK (2000). The inhibitory action of spices against pathogens that might be capable of growth in a fish sauce (Mehiawah) from the Middle East. *International Journal of Food Microbiology*, **57**: 129-133.

Anonymous (2006). *Emblica officinalis*. <http://www.ayurvedicure.com/amla.htm>.

Bhattacharya A, Chatterjee A, Ghosal S and Bhattacharya SK (1999). Antioxidant activity of active tannoid principles of *Emblica officinalis* (amla). *Indian J. Exp. Biol.*, **37**: 676-680.

Chaudhry NMA and Tariq P (2006). Bactericidal activity of black peeper, bay leaf, aniseed and coriander against oral isolates. *Pak. J. Pharm. Sci.*, **19**: 214-218.

Chithra V and Leelamma S (1997). Hypolipidemic effect of coriander seed (*Coriandrum sativum*): mechanism of action. *Plant Foods Hum. Nutr.*, **51**(2); 167-172.

Chopra D and Simon D (2000). The Chopra Centre Herbal Handbook. Rider, London.

DeSouza EL, Stamford TLM, Lima EO, Tarajano VN and Filho JBM (2005). Antimicrobial effectiveness of spices: an approach for use in food conservation system. *Braz. Arch. Biol. Technol.*, **48**(4): 1516-8913.

Dutta BK, Rahman I and Das TK (1998). Antifungal activity of Indian plant extracts. *Mycoses*, **41**(11-12): 535-536.

Gales CA, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML, Pfaller MA, Doern GV and the SENTRY Study Group (Latin America) (2000). Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial survival program. *J. Antimicrob. Chemotherap.*, **45**: 295-303.

- Ghosal S, Tripathi VK and Choun S (1996). Active constituents of *Emblca officinalis*: Part 1. The chemistry and antioxidant effect of two new hydrolysable tannins. Emblicanin A and B. *Indian J. Chem.*, **35B**: 941-948.
- Gray AM and Flatt PR (1999). Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant *Coriandrum sativum* (coriander). *Br. J. Nutr.*, **81**(3): 203-209.
- Hvidberg H, Struve C, Krogfelt KA, Christensen N, Rasmussen SN and Frimodt-Moller N (2000). Development of long-term ascending urinary tract infection mouse model for antibiotic treatment studies. *Antimicrob. Agents Chemother.*, **44**: 156-163.
- Jeena KL, Joy KL, and Kuttan R (1999). Effects of *Emblca officinalis*, *Phyllanthus amarus* and *Picrorhiza kurroa* on N-nitrosodiethyl amine induced hepatocarcinogenesis. *Cancer Lett.*, **136**: 11-16.
- Jose KJ and Kuttan R (2000). Hepatoprotective activity of *Emblca officinalis* and Chyavanprash. *Journal of Ethnopharmacology*, **72**(1-2): 135-140.
- Jose JK, Kuttan Y, and Kuttan R (2001). Antitumor activity of *Emblca officinalis*. *J. Ethnopharmacol.*, **75**: 65-69.
- Khanna P and Nag TH (1973). Isolation, identification and screening of phyllemblics from *Emblca officinalis* Gaertn. tissue culture. *Indian Journal of Pharmacy*, **35**(1): 23-28.
- Kim HJ, Yokozawa T, Kim HY, Tohda C, Rao TP and Juneja LR (2005). Influence of amla (*Emblca officinalis* Gaertn.) on hypocholesterolemia and lipid peroxidation in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol.*, **51**(6): 413-418.
- Kubo I, Fujita K, Kubo A, Nihei K and Ogura T (2004). Antibacterial activity of coriander volatile compounds against *Salmonella choleraesuis*. *J. Agric Food Chem.*, **52**(11): 3329-3332.
- Noor N, Ajaz M, Rasool SA and Pirzada ZA (2004). Urinary tract infections associated with multi-drug resistant enteric bacilli: characterization and genetical studies. *Pakistan Journal of Pharmaceutical Sciences*, **17**(2): 115-123.
- Parrotta AJ (2001). Healing plants of Peninsular India. New York: CABI Publishing, p.308.
- Perianayagam JB, Narayan S, Gnanasekar G, Pandurangan A, Raja S, Rajagopal K, Rajesh R, Vijayarakumar P and Vijaykumar SG (2005). Evaluation of antidiarrheal potential of *Emblca officinalis*. *Pharmaceutical Biology*, **43**(4): 373-377.
- Rege NN, Thatte UM, and Dahanukar SA (1999). Adaptogenic properties of six Rasayana herbs used in Ayurvedic medicine. *Phytother Res.*, **13**: 275-291.
- Saeed S and Tariq P (2005). Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pakistan Journal of Botany*, **37**(4): 997-1001.
- Saeed S and Tariq P (2006). Effects of some seasonal vegetables and fruits on the growth of bacteria. *Pakistan Journal of Biological Sciences*, **9**(8): 1547-1551.
- Sairam K, Rao CV, Dora BM, Vijay KK, Agarwal VK, and Goel RK (2002). Antiulcerogenic effect of methanolic extract of *Emblca officinalis*: An experimental study. *J. Ethnopharmacol.*, **82**: 1-9.
- Scartezzini P, Antognoni F, Raggi MA, Poli F and Sabbioni C (2006). Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblca officinalis* Gaertn. *J. Ethnopharmacol.*, **104**(1-2): 113-118.
- Sharma SK, Perianayagam BJ, Joseph A, Christina AJM (2003). Antiinflammatory activity of ethanol aqueous extracts of *Emblca officinalis* Gaertn fruits. *Hamdard Medicus*, **46**: 75-78.
- Waheed A, Miana GA, Ahmad SI and Khan MA (2006). Clinical investigation of hypoglycemic effect of *Coriandrum sativum* in type-2 (NIDDM) diabetic patients. *Pakistan Journal of Pharmacology*, **23**(1): 7-11.

Received: 23-09-2006 – Accepted: 14-02-2007