Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 3 (3): 310-314, 2004
ISSN 1682-3974

Screening for Antibacterial Properties of Some Iranian Plants Against Two Strains of *Escherichia coli*

G.H. Shahidi Bonjar
Department of Plant Pathology, College of Agricultural Sciences, Bahonar University of Kerman, Iran

Abstract: Plants used in folkloric medicine of Iranian native people were collected from Southeast regions of Iran. Methanol extracts were prepared and evaluated in a test against two strains of *Escherichia coli*. From 180 plant species in 72 families, 17 samples in 11 families showed anti-*E. coli* activity. At 20 mg ml^{-1} concentration, the most active plants with diameter of inhibition zones of 12 mm or more on both bacterial strains were *Lawsonia inermis*, *Cinnamomum zeylanicum* and *Dianthus caryophyllus*. *Trigonella foenum-graecum*, *Cuminum cyminum*, *Alhagi maurorum*, *Apium graveolens*, *Colchicum luteum*, *Origanum majorana*, *Calendula officinalis*, *Nepeta racemosa* and *Coriandrum sativum* were active only against *E. coli* (PTCC No. 1330) and *Camellia sinensis* and *Anthemis nobilis* were active only against *E. coli* (PTCC No. 1338). The lowest minimum inhibitory concentration found in *Trigonella foenum-graecum* as 0.46 mg ml^{-1}. All of the active extracts were well stable at room temperature in DMSO: methanol (1:1, v/v) solvent and dry state up to 18 months and did not show any reduction of activity against the sensitive bacterial strains.

Key words: Antibacterial, plant extracts, Iranian folkloric medicine, *Escherichia coli*

INTRODUCTION

According to the reports of many researchers[1,2], antibacterial resistance is a worldwide growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world. One of the measures to combat the increasing rate of resistance in long run, is to have continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute with no-effective ones. Natural resources specially plants and microorganisms are potent candidates for this aim. Usage of plants in curing illnesses has deep roots in man's history since plants are sources of many life-sustaining metabolites. Traditional curative medicine of most nations is based upon the plants. Ethnobotanical uses of plants prevail among Iranian native people (INP). They use plants in treating burns, dermatophytes and infectious diseases. In many of these communities, infectious urinary diseases are cured by using filtrates of macerated plants orally. The curative plants vary between these natives in different localities. In order to determine inhibitory effects of the plants against the most prevalent urinary tract pathogen, *Escherichia coli*, a two year study performed to screen most of the medicinal plants used by INP against two strains of this pathogen. According to the information gathered about the ethnobotanical usages, the plant organs used in this study are as used by INP. *E. coli* are facultative anaerobes in the normal intestinal flora of human and animals[3,4] however, pathogenic strains of these bacteria are an important cause of bacterial infections. In humans, these strains are the foremost cause of urinary tract infections[5], as well as a major cause of neonatal meningitis[6], nosocomial septicemia and surgical site infections[7]. Infection with Shiga toxin-producing *E. coli* may also result in complications including thrombocytopenic purpura, severe hemorrhagic colitis and hemolytic uremic syndrome[8]. Recent reports have proposed that the use of tetracyclines, sulfa drugs, cephalosporins and penicillins to be a major factor in the emergence and dissemination of antimicrobial-resistant *E. coli*[9-14]. This bacterium is also a major pathogen of worldwide importance in commercially produced poultry, contributing significantly to economic losses in both chickens and turkeys[15]. Some researchers have expressed that antimicrobial resistance in *E. coli* can be transferred to humans from food or companion animals[16-18] hence these animals can serve as important reservoirs of antimicrobial-resistant *E. coli*. Not to loose the battle in the war against antimicrobial-resistant bacteria, one major way is to extend man's knowledge about new antimicrobial sources. In this regard, many other researchers have also reported detection of antibacterials from plants.

Corresponding Author: G.H. Shahidi Bonjar, Department of Plant Pathology, College of Agricultural Sciences, Bahonar University of Kerman, Iran Email: shahidi@mail.uk.ac.ir

McCutcheon *et al.*[19] tested 100 methanol extracts of the plants, used by British Colombian native people, against 11 bacterial isolates. They found 85% of the plants were active at least against one of the bacteria. Pedersen *et al.*[20] examined 27 medicinal plant extracts of Rubiaceae and found 11 of them with antibacterial activity. Mansouri *et al.*[21] evaluated antibacterial activity of crude extract of *Myrtus communis* against 10 laboratory strains of bacteria. They noticed that the crude extract inhibited the growth of all tested bacteria except *Campylobacter jejuni*. Yoshikazu *et al.*[22] evaluated inhibitory effect of 21 plant samples on production of Verotoxin by enterohemorrhigic *E. coli* 0157:H7, a food-borne human pathogen and found 4 plants well active. In the present research, inhibitory effects of 180 plant samples in 72 families were investigated against *E. coli*.

MATERIALS AND METHODS

Plant material and extraction procedure: One hundred and eighty plant species belonging to 72 families, being used by INP, were collected from Southeast regions of Iran and identified by Mrs. P. Rashid Farrokhi in the Herbarium of Plant Systematic Laboratory of the College of Agricultural Sciences, Bahonar University of Kerman, Iran. Some of the plants used by INP but not grown in Iran or were not found in the collection regions, were obtained from the mentioned Herbarium. Voucher specimens of all plants were preserved and kept there with a voucher specimen No. The fine powder of air dried specimens were extracted three times with methanol at 70°C for 4 h and the extracts were then concentrated under reduced pressure to yield a dense residue. Each sample transferred to glass vials and lyophilized overnight before use.

Test organisms and agar well diffusion bioassay: From Persian Type Culture Collection, Tehran, Iran (PTCC), two registered strains namely, *Escherichia coli* (PTCC No. 1330) and *E. coli* (PTCC No. 1338) were obtained. The bacteria were rejuvenated on Mueller-Hinton-Agar medium (MH, E. Merk, Germany) and subcultured as needed. For bioassays, suspension of approximately $1.5x10^6$ cells ml^{-1} in sterile normal saline were prepared as described by Forbes *et al.*[37] and about 1.5 ml of it was uniformly seeded on MH in 12x1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. Concentration of 20 mg ml^{-1} of each extract was prepared in dimethyl sulfoxide: methanol (1:1, v/v) solvent (DM solvent) and administered to fullness in each well. Culture plates, were incubated at 37°C for 48 h. Bioactivity was determined by measuring diameter of inhibition zones (DIZ) in mm. Solvent controls were included, although no antibacterial activity has been noted in the solvent employed. All samples tested in triplicate and average results recorded.

Determination of minimum inhibitory concentration: Minimum inhibitory concentrations (MIC) of the most active methanol extracts were determined using two-fold serial dilutions of 15 to 0.46 mg ml^{-1} in DM solvent against both strains of *E. coli* in Agar well diffusion method as mentioned earlier.

Determination of shelf life or stability of the crude extracts: To measure the stability of the bioactive extracts in solubilized and dry states, 20 mg ml^{-1} of each was prepared in DM solvent and 20 mg dry samples in small

Table 1: Antibacterial activity, indicated by diameter of inhibition zones (DIZ, mm), of plants used in Iranian traditional medicine against two strains of *Escherichia coli*. Plants are listed according to their relative activity. Blanks represent no inhibition and are equal to zero

b	a	tp	Common name	Plant family	Plant species
12	17	LE	Henna	Lythraceae	*Lawsonia inermis* L. Syn. *L. alba* L.
12	14	SB	Cinnamon	Lauraceae	*Cinnamomum zeylanicum* Bl.
12	12	WP	Carnation	Caryophyllaceae	*Dianthus caryophyllus* L.
10	10	LE	Jujuba	Rhamnaceae	*Ziziphus zizyphus* (L.) Karst. Syn. *Ziziphus jujube* (L.) Karst.
10	9	SE	-	Apiaceae	*Trachyspermum ammi* (L.) Link. Syn. *Ammi copticum; Carum copticum*
10	9	RO	Sultan Zamback	Liliaceae	*Lilium candidum* West.
	18	SE	Siclefruit fenugreek	Papilionaceae	*Trigonella foenum-graecum* L.
	17	FR	Cumin	Apiaceae	Cuminum cyminum L.
	17	SG	Camel thorn	Papilionaceae	*Alhagi maurorum* Medik.
	12	LE	Celeriac	Apiaceae	*Apium graveolens* (Miller) Gaudin. Syn. *Apium rapaceum*
	12	WP	Yellow autumn crocus	Colchicaceae	*Colchicum luteum* Bak.
	12	LE	Sweet marjoram	Labiatae	*Origanum majorana* L. Syn. *Majorana hortensis;* M. majorana
	11	FL	Marigold	Compositae	Calendula officinalis L.
	11	RO	-	Labiatae	*Nepeta racemosa* Lam.
	10	SE	Coriander	Apiaceae	*Coriandrum sativum* L.
10		LE	Tea plant	Theaceae	*Camellia sinensis* (L.) Kuntze.
10		FL	Camomile	Compositae Syn. *Chamaemelum nobile*	*Anthemis nobilis* (L.) All.

tp: tested parts as FL: flower, FR: fruit, LE: leaves, RO: roots, SB: stem bark, SE: seeds, SG: stem gum and WP: whole plant. a : diameter of inhibition zones (mm) against *Escherichia coli* (PTCC No. 1330) and b : against *E. coli* (PTCC No. 1338)

Table 2: Minimum inhibitory concentration (MIC) of the most active plant extracts against two strains of *Escherichia coli*. Plants are listed according to their relative activity. Concentration at which diameter of inhibition zone (DIZ, mm) is indicated in bold represents the MIC. Blanks represent no inhibition and are equal to zero

Mg ml^{-1} 0.46		0.93		1.87		3.75		7.5		15		
b	a	b	a	b	a	b	a	b	a	b	a	Plant species
					10		12	9	14	10	15	*Lawsonia inermis* L.
								8	9	10	12	*Cinnamomum zeylanicum* Bl.
								8	8	10	10	*Dianthus caryophyllus* L.
										9	9	*Ziziphus zizyphus* (L.) Karst.
										9	9	*Trachyspermum ammi* (L.) Link.
										8		*Lilium candidum* West.
	9		9		11		13		15		17	*Trigonella foenum-graecum* L.
					10		13		16		16	Cuminum cyminum L.
							9		11		14	*Alhagi maurorum* Medik.
												Apium graveolens (Miller) Gaudin
									8		10	*Colchicum luteum* Bak.
												Origanum majorana L.
											9	Calendula officinalis L.
											10	*Nepeta racemosa* Lam.
											8	*Coriandrum sativum* L.
												Camellia sinensis (L.) Kuntze.
										9		*Anthemis nobilis* (L.) All.

a, diameter of inhibition zones (mm) against *Escherichia coli* (PTCC No. 1330); b, against *E. coli* (PTCC No. 1338)

vials. The samples kept at room temperature and tested for antibacterial activity against the sensitive strain of *E. coli* at 14 days intervals up to 18 months.

RESULTS

From 180 plant species in 72 families, only 17 species in 11 families showed anti-*E. coli* activity as presented in Table 1. At 20 mg ml^{-1} concentration, the most active plants with DIZ of 12 mm or more on both bacterial strains were *Lawsonia inermis*, *Cinnamomum zeylanicum* and *Dianthus caryophyllus*. *Trigonella foenum-graecum*, *Cuminum cyminum*, *Alhagi maurorum*, *Apium graveolens*, *Colchicum luteum*, *Origanum majorana*, *Calendula officinalis*, *Nepeta racemosa* and *Coriandrum sativum* were active only against *E. coli* (PTCC No. 1330) and *Camellia sinensis* and *Anthemis nobilis*, were active only against *E. coli* (PTCC No. 1338). Apiaceae with four active plants had the highest number of actives. The lowest MIC found in *Trigonella foenum-graecum* as 0.46 mg ml^{-1} and in *L. inermis*, *C. zeylanicum* and *D. caryophyllus* as 7.5 mg ml^{-1} as indicated in Table 2. Considering shelf life of the samples, all of the active extracts were stable at room temperature in both DM solvent and dry state up to 18 months and did not show any reduction of activity against the sensitive strain of *E. coli*.

DISCUSSION

The emergence and dissemination of antibacterial resistance is well documented as a serious problem worldwide[23,1,2]. Smith *et al.*[24] express that "The emergence of bacterial resistance threatens to return us to the era before the development of antibiotics". Fridkin *et al.*[25] reported antimicrobial resistance increasing in all health-care-associated pathogens. They examined changes in resistance dissemination during 1996-1999 in 23 hospitals and noticed significant increase in prevalence of resistance. The prevalence of antimicrobial resistance in urinary pathogens has been well demonstrated by several workers. Gupta *et al.*[26] studying acute cystitis in women, reported prevalence of multi-resistant *E. coli* in more that 20% of 4342 urine isolates to ampicillin, cephalothin and sulfamethoxazole in each year of their study. They noticed that the prevalence of resistance to trimethoprim and trimethoprim-sulfamethoxazole rose from 9% in 1992 to more than 18% in 1996 among *E. coli* isolates. In a 4 year period, Manges *et al.*[27] examined 255 *E. coli* isolates of three geographically diverse communities, California, Michigan and Minnesota and noticed that a single clonal group of *E. coli* accounted for nearly half of community-acquired urinary tract infections in women that were caused by *E. coli* strains with resistance to trimethoprim-sulfamethoxazole. Schroeder *et al.*[28] by investigating 752 *E. coli* isolates found that approximately 40% of human isolates were resistant to trimethoprim-sulfamethoxazole. In poultry industry, prevalence of resistance in *E. coli* is uprising too. Blanco *et al.*[15] reported that from 468 avian *E. coli* strains isolated in Spain, 67% showed resistance to trimethoprim+sulfamethoxazole and 13 to 24% showed resistance to fluoroquinolones. The perspective of rapid emergence of drug resistance among bacterial pathogens

shows that the potencies of prevalent antibiotics are decreasing steadily, leading to reduced useful-period of drugs. This situation implies the need for new and safe antimicrobials for replacement with invalidated antimicrobials or use in antibiotic rotation programs[29-31]. Although the nature and number of active antibacterial principles involved in each extract of the present research are not clear, but the activity of several plant-extracts against the *E. coli* strains, opens new gates for future studies. I suggest evaluation of the active plants extracts presented here against some of the other drug-resistant problematic bacteria like vancomycin-resistant enterococci and *E. coli* strains with resistance to trimethoprim-sulfamethoxazole, two of the most problematic bacteria-in terms of their occurrence and impact on the clinical outcomes of patients[32-36]. In addition, I propose use of these plants in further evaluation as poultry water or feed-additives for prevention or reduction of *E. coli* or some other bacterial infections. Sensitivity results of the two *E. coli* strains show that they have different sensitivity patterns to the tested plants. Accordingly, the plants having activity against both strains are of more interest for further evaluation.

ACKNOWLEDGMENTS

Financial and moral support of Research Affairs Office of Bahonar Univ. of Kerman is appreciated. Thanks to helpful work of Mrs. Parvin Rashid Farokhi and her colleagues on scientific diagnosis of the plants. The results of this research are dedicated to Mr. A. Afzalipour the founder of Bahonar Univ. of Kerman. God may bless him.

REFERENCES

1. Cohen, M.L., 2002. Changing patterns of infectious disease. Nature, 406: 762-767.
2. WHO, 2001. WHO Global Strategy for Containment of Antimicrobial Resistance. Available on Internet at: www.who.int/emc-documents/ antimicrobial_resistance/docs/EGlobal_Strat.pdf.
3. Bonten, M., E. Stobberingh, J. Philips and A. Houben, 1990. High prevalence of antibiotic resistant *Escherichia coli* in faecal samples of students in the south-east of The Netherlands. J. Antimicrob. Chemother., 26: 585-592.
4. Conway, P. and G. Macfarlane, 1995. Microbial Ecology of the Human Large Intestine. In: 'Human Colonic Bacteria: Role in nutrition, physiology and pathology'. (Eds. G. Gibson and G. MacFarlane), CRC Press, London, pp: 1-24.
5. Falagas, M. and S. Gorbach, 1995. Practice guidelines: Urinary tract infections. Infect. Dis. Clin. Pract., 4: 241-257.
6. Klein, J.O., R.D. Feigin and G.H. McCracken Jr., 1986. Report of the task force on diagnosis and management of meningitis. Pediatrics, 78: 959-982.
7. Thielman, N.M. and R.L. Guerrant, 1999. *Escherichia coli*. In: 'Antimicrobial Therapy and Vaccines'. (Eds. V.L. Yu, T.C. Merigan Jr. and S.L. Barriere), Williams and Wilkins Co: Baltimore, pp: 188-220.
8. Griffin, P.M., 1995. *Escherichia coli* O157:H7 and other Enterohemorrhagic *Escherichia coli*. In: 'Infections of the Gastrointestinal Tract'. (Eds. M.J. Blaser, P.D. Smith, J.L. Radvin, H.B. Greenberg and R.L. Guerrant). Raven Press, New York, pp: 739-761.
9. Galland, J.C., D.R. Hyatt, S.S. Crupper and D.W. Acheson, 2001. Prevalence, antibiotic susceptibility and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Appl. Environ. Microbiol., 67: 1619-1627.
10. Meng, J., S. Zhao, M.P. Doyle and S.W. Joseph, 1998. Antibiotic resistance of *Escherichia coli* O157:H7 and O157: NM isolated from animals, food and humans. J. Food. Prot., 61: 1511-1514.
11. Schroeder, C.M., C. Zhao, C. DebRoy, J. Torcolini, S. Zhao and D.G. White, 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine and food. Appl. Environ. Microbiol., 68: 576-581.
12. Stephan, R. and S. Schumacher, 2001. Resistance patterns of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from animals, food and asymptomatic human carriers in Switzerland. Lett. Appl. Microbiol., 32: 114-117.
13. Teshager, T., I.A. Herrero, M.C. Porrero, J. Grade, M.A. Moreno and L. Dominguez, 2000. Surveillance of antimicrobial resistance in *Escherichia coli* strains isolated from pigs at Spanish slaughterhouses. Int. J. Antimicrob. Agents, 15: 137-142.
14. Van Den Bogaard, A.E., N. London, C. Driessen and E.E. Stobberingh, 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. J. Antimicrob. Chemother., 47: 763-771.
15. Blanco, J.E., M. Blanco, A. Mora and J. Blanco, 1997. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. J. Clin. Microbiol., 35: 2184-2185.

16. Zhao, S., D.G. White, B. Ge, S. Ayers, S. Friedman and L. English, 2001. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Appl. Environ. Microbiol., 67: 1558-1564.
17. Winokur, P.L., D.L. Vonstein, L.J. Hoffman, E.K. Uhlenhopp and G.V. Doern, 2001. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob. Agents Chemother., 45: 2716-2722.
18. Fey, P.D., T.J. Safranek, M.E. Rupp, E.F. Dunne, E. Ribot and P.C. Iwen, 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N. Engl. J. Med., 432: 1242-1249.
19. McCutcheon, A.R., S.M. Ellis, R.E. Hancock and G.H. Towers, 1992. Antibiotic screening of medicinal plants of the British Colombian native people. J. Ethnopharmacol., 37: 212-223.
20. Pederson, O., A. Gurib-fakim, H. Subratty and A. Andersen, 1999. Pharmaceutical properties of seven medicinal plants of Rubiaceae from Mauritius. Pharm. Biol., 37: 202-207.
21. Mansouri, S., A. Foroumadi, T. Ghanei and A. Gholamhosseinian Najar, 2001. Antibacterial activity of the crude extracts and fractionated constituents of *Myrtus Communis*. Pharm. Biol., 39: 399-401.
22. Yoshikazu, S., H. Murata, T. Nakanishi and Y. Inatomi, 2001. Inhibitory effect of plant extracts on production of Verotoxin by enterohemorrhagic *Escherichia coli* O157 : H7. J. Health Sci., 47: 473-477.
23. Gold, H.S. and R.C. Moellering, 1996. Antimicrobial-drug resistance. N. Engl. J. Med., 335: 1445-1453.
24. Smith, T.L., M.L. Pearson and K.R. Wilcox, 1999. Emergence of vancomycin resistance in *Staphylococcus aureus*. N. Eng. J. Med., 340: 493-501.
25. Fridkin, S.K., H.A. Hill, N.V. Volkava, J.R. Edwards, R.M. Lawton and R.P. Gaynes, 2002. Temporal changes in prevalence of antimicrobial resistance in 23 U.S. hospitals. Emer. Infec. Dis., 8: 697-700.
26. Gupta, K., D. Scholes and W.E. Stamm, 1999. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. JAMA., 281: 736-738.
27. Manges, A.R., J.R. Johnson, B. Foxman, T.T. O'Bryan, K.E. Fullerton and L.W. Riley, 2001. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. N. Engl. J. Med., 345: 1007-1013.
28. Schroeder, C.M., L. Meng, S. Zhao, C. DebRoy, J. Torcolini and C. Zhao, 2002. Antimicrobial resistance of *Escherichia coli* O26, O103, O111, O128 and O145 from animals and humans. Emer. Infec. Dis., 8: 1409-1414.
29. Niederman, M.S., 1997. Is "crop rotation" of antibiotics the solution to a "resistant" problem in the ICU? Am. J. Resp. Crit. Care Med., 156: 1029-1031.
30. Gerding, D.N., T.A. Larson and R.A. Hughes, 1991. Aminoglycoside resistance and aminoglycoside usage: ten years of experience in one hospital. Antimicrob. Agents Chemother., 35: 1284-1290.
31. Quale, J., D. Landman and E. Atwood, 1996. Experience with a hospital-wide outbreak of vancomycin-resistant enterococci. Am. J. Infect. Cont., 24: 372-379.
32. Lelievre, H., G. Lina, M.E. Jones, C. Olive, F. Forey and M. Roussel-Delvallez, 1999. Emergence and spread in French hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and other antibiotics. J. Clin. Microbiol., 37: 3452-3457.
33. O'Brien, F.G., J.W. Pearman and M. Gracy, 1999. Community strain of methicillin-resistant *Saphylococcus aureus* involved in a hospital outbreak. J. Clin. Microbiol., 37: 2858-2862.
34. Abi-Hanna, P., A.L. Frank and J.P. Quinn, 2000. Clonal features of community-acquired methicillin-resistant *Saphylococcus aureus* in children. Clin. Infect. Dis., 30: 630-631.
35. Cookson, B.D., 2000. Methicillin-resistant *Staphylococcus aureus* in the community. New battlefronts, or are the battles lost? Control. Hosp. Epidemiol., 21: 398-403.
36. Shopsin, B., B. Mathema and J. Martinez, 2000. Prevalence of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in the community. J. Infect. Dis., 182: 359-362.
37. Forbes, B.A., D.F. Sahm, A.S. Weissfeld and E.A. Trevino, 1990. Methods for testing antimicrobial effectiveness. In: ' Bailey and Scott's Diagnostic Microbiology'. (Eds E.J. Baron, L.R. Peterson and S.M. Finegold), Mosby Co: St Louis, Missouri, pp: 171-194.