Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

EFFECT OF CARROT AND GINGER EXTRACTS (ALONE AND IN COMBINATION WITH CIPROFLOXACIN) ON MULTIDRUG RESISTANT *PSEUDOMONAS AERUGINOSA*

Al-Alak, Shaymaa Khudhr; Ali, Wala'a Shawkat*; Kadum, Maha Mikhlef and Othman, Nabaa Abd-Alkhalik
Department of Biology, College of Science, Al-Mustansiriya University, P. O. box 14022, Baghdad, Iraq
*Corresponding Author Email: microrose.2788@yahoo.com
DOI: 10.7897/2277-4572.0320121
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 05/02/14 Revised on: 23/03/14 Accepted on: 11/04/14

ABSTRACT
Antibacterial activity of ginger (*Zingiber officinale*) and carrot (*Daucus carota*) watery extracts (cold and hot) with or without ciprofloxacin against 53 isolates of multidrug- resistant *Pseudomonas aeruginosa* was screened by agar well diffusion method. The results showed that the hot watery extracts were more active than cold watery extracts. The combination of ciprofloxacin and hot watery extracts of ginger or carrot was most effective than ciprofloxacin alone or plant extracts alone, also the results showed that the mixture of ciprofloxacin and ginger extract was the best for inhibition growth of *P. aeruginosa*. These results indicate that the plant extracts contain natural inhibitors and the combination between them and ciprofloxacin could be useful in fighting emerging drug-resistance *P. aeruginosa.*
Keywords: Antibacterial activity, *Pseudomonas aeruginosa*, ginger extract, carrot extract.

INTRODUCTION

Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria[1]. Recently different authors have reported the urgent need for new antimicrobial agents to replenish the arsenal of anti-infective agents. In fact, during the last few years, medicinal plants have attracted the attention of pharmaceutical and scientific communities, and evidence has demonstrated the promising potential of antimicrobial plant-derived substances. The antimicrobial effect of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies[2]. One approach to treat infectious diseases is the use of plant extracts individually and /or as an alternative approach is the use of combination of antibiotics with plant extracts. This latter approach i.e. combination therapy or synergistic therapy; against resistant microorganisms may lead to new ways of treating infectious diseases and probably this represents a potential area for further future investigations. Combination therapy is helpful and useful for patients with serious infections caused by drug resistant pathogens[1]. Ginger is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice[3]. In many parts of the world, *Z. officinale* has medicinal and culinary values[4]. Fresh ginger has been used for cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, less of appetite, and rheumatism[5]. Ginger compounds are active against a form of diarrhea which is the leading cause of infant death in developing countries. Zingerone (vanillylacetone) is likely to be the active constituent against enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea[6]. The genus Daucus (Apiaceae) comprises weedy plants of about 60 species, widely distributed and commonly cultivated for their fleshy edible roots. *Daucus carota* L. ssp. *carota* (wild carrot)[7] is natural food rich in carbohydrate, carotene, vitamins, minerals and others, this plant used in many foods as fresh or boiled especially in the preparation of broths with onion juices and a juices in which the carrot juices considered as one of the healthy drink, which is beneficial for humans particularly in fresh state as alone or mixed with fruits or milk[8]. It is used as antibacterial, stimulant, antiseptic, carminative, diuretic, hepatoprotective, anti steroidogenic, anti-inflammatory and in treatment of jaundice and stomach disorders[7] and also can be used as activator for gastrointestinal tract and in treatment of dermatitis[8]. Ciprofloxacin, a member of the large and widely used fluoroquinolone group of antimicrobial drugs, is considered the empirical treatment of choice of gastrointestinal infections in adults[9]. For Gram-negative infections, most *in vitro* evidence shows that fluoroquinolones have better activity than other antibiotics in killing and preventing attachment of slow-growing biofilm-associated organisms[10]. *Pseudomonas aeruginosa* is an important bacterial pathogen, particularly as a cause of infections in hospitalized patients, immune compromised hosts and patients with cystic fibrosis[11]. It has been identified as the 2nd most frequent organism causing ventilator associated pneumonia, the 4th most common causing catheter-associated urinary tract infections, the 5th cause of surgical site infections and the 7th cause of central-line-associated bloodstream infections[12]. It causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments and resistance to many antibiotics and antiseptics. Infections caused by this microorganism are often sever, life threatening and difficult to be treated[13]. In Iraq ciprofloxacin is widely used to treat many bacterial infections and one of them is *P. aeruginosa* infections therefore, this study was aimed to evaluate the antibacterial activity of ginger and carrot watery extracts with or without ciprofloxacin against multidrug-resistant *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Bacterial isolates and culture media

A total of 53 multidrug- resistant *P. aeruginosa* isolates were obtained from the department of biology, college of science, Al-Mustansiryia University, Baghdad, Iraq. Mueller Hinton agar (MHA) was provided from Oxoid (England).

Plant materials

The ginger and carrot were obtained from the local Market in Baghdad and the extracts were prepared as follow: The ginger and carrot were washed, peeled, cut into pieces and ground using an electric blender. 25 g of the ground material were dissolved in 100 ml of boiling and cold sterile distilled water, separately and left for 24 h at room temperature with occasional shaking then filtered to obtain clear infusion[14] and the filtrates were stored at 4°C until used.

Antibacterial activity of plant extracts

Filtrates of ginger and carrot were diluted with sterile distilled water in a ratio 1:1, separately. Antibacterial activity of crude and diluted filtrates were assayed using agar well diffusion method[14], MHA plates were prepared and seeded with 10^5cell/ml of overnight cultures of *P. aeruginosa* isolates. Wells of 6mm in diameter were made using a sterile cork borer then filled with 100 µl of plant filtrates and incubated at 37°C for 24 h. Wells without plant filtrates were served as control. Inhibition was detected by a zone of clearing around the filtrate well.

Minimum inhibitory concentration (MIC) of ciprofloxacin

Minimum inhibitory concentration (MIC) of ciprofloxacin (Dolder/Switzerland) was determined against the most sensitive isolates of *P. aeruginosa* by an agar dilution method as described by Clinical and Laboratory Standards Institute (CLSI)[15]. The range of dilutions was 0.5 to 1024 µg/ml. 5 µl of each bacterial isolate was spotted on the MHA surface by micropipette, the test plates were incubated at 37°C for 18 h. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism.

Antibacterial activity of plant extracts and ciprofloxacin

Crude Filtrates with boiling water of ginger and carrot were mixed with ciprofloxacin (MIC concentration) in a ratio 1:1, separately and the antibacterial activity of them was assayed by agar well diffusion method as described above.

RESULTS AND DISCUSSION

The antibacterial activity of watery extracts of ginger and carrot was evaluated against *P. aeruginosa* isolates and the results showed that the crude extracts of them with boiling water were more effective than the cold watery extracts (crude and diluted) which appeared no effect on *P. aeruginosa* isolates. 83 % of the isolates were sensitive to crude extract of ginger which recorded inhibition zones ranged from 10 mm to 23 mm for these isolates (Figures 1 and 2). The antibacterial activity of ginger extract could be attributed to the chemical properties of ginger. The main constituents of ginger are sesquiterpenoids with zingiberene as the main component. Other components include β-sesquiphellandrene, bisabolene and farnesene, which are sesquiterpenoids, and monoterpenoid (β-sesquiphellandrene, cineol and citral) fractions in traces[16]. On the other hand, only 44 % of the isolates were sensitive to crude extract of carrot which recorded inhibition zones ranged from 7 mm to 20 mm (Figures 1 and 2). Ahmed *et al*.[7] found that the roots of the wild *Daucus carota* ssp. *Carota* contain a range of low antibacterial activities against four gram positive (*Staphylococcus aureus*, *Streptomyces scabies*, *Bacillus subtilus*, *Bacillus cereus*) and two gram negative species (*Pseudomonas aeruginosa*, *Escherichia coli*).

The effect of carrot extracts on the inhibition of growth of *P. aeruginosa* related to many factors as plant genus, the period of ripening, type of the soil, climatic factors and the chemical structure of the extracts also to the presence of the active ingredient in the extract and to the type of microorganism affected by the plant extracts[8]. The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, types of microorganism, composition of the food, pH value and temperature of the environment. However, still there is little information about the exact mechanism of their antimicrobial action[3].

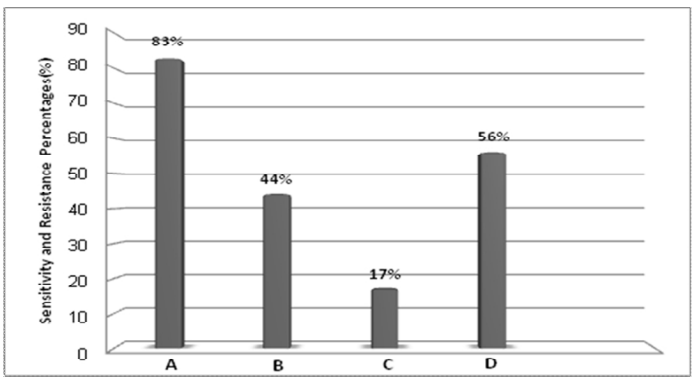

Figure 1: A and B, Sensitive Percentages of *Pseudomonas aeruginosa* Isolates to Watery Crude Extracts of Ginger and Carrot, Respectively. C and D, Resistance Percentages of *Pseudomonas aeruginosa* Isolates to Watery Crude Extracts of Ginger and Carrot, Respectively

Minimum inhibitory concentration (MIC) of ciprofloxacin was determined against *P. aeruginosa* by an agar dilution method and the results showed that the MIC was 128 µg/ml. The combination of ciprofloxacin (MIC concentration) and crude extract of ginger or carrot was determined and the results showed that the mixture of ciprofloxacin and crude extract of ginger or carrot was more active against *P. aeruginosa* isolate comparison with ciprofloxacin alone or crude extract alone. Ciprofloxacin and crude extract of ginger recorded inhibition zones ranged from 30 mm to 56 mm,

ciprofloxacin and crude extract of carrot recorded inhibition zones ranged from 22 mm to 35 mm, while ciprofloxacin alone recorded inhibition zones ranged from 12 mm to 35 mm (Figure 2). The ability of plant extracts to potentiate antibiotics has not been well explained. It is predicted that inhibition of drug efflux and alternative mechanisms of action could be responsible for the synergistic interactions between plant extracts and antibiotics[13]. In conclusion, our results revealed that the combination of plant extracts and ciprofloxacin could be useful in fighting emerging drug-resistance problem and *in vivo* experiments are needed to confirm pseudomonal protection using these combinations.

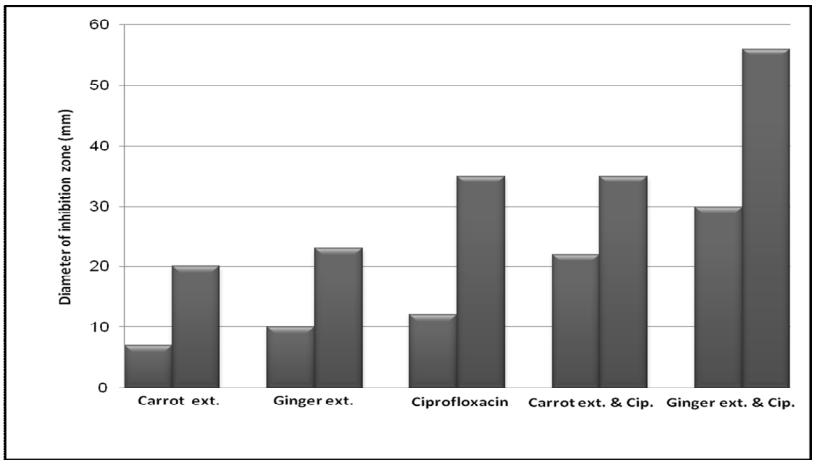

Figure 2: Minimum and Maximum Diameters of Inhibition Zones (mm) for Ciprofloxacin Alone, Watery Crude Extracts of Carrot (Alone and With Ciprofloxacin) and Ginger (Alone and With Ciprofloxacin) Against *Pseudomonas aeruginosa* Isolates

REFERENCES

1. Chanda S, Rakholiya K. Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advances; 2011. p. 520-529.
2. Savoia D. Plant –derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol 2012; 7(8): 979–990. http://dx.doi.org/10.2217/fmb.12.68
3. Akintobi OA, Onoh CC, Ogele JO, Idowu AA, Ojo OV, Okonko IO. Antimicrobial activity of *Zingiber officinale* (ginger) extract against some selected pathogenic bacteria. Nature and Science 2013; 11(1): 7-15.
4. Omoya FO, Akharaiyi FC. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. Int Res J of Pharmaceuticals 2012; 2(5): 127-132.
5. Karuppiah P, Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific J of Tropical Biomedicine; 2012. p. 597-601.
6. Chen JC, Huang LJ, Wu SL, Kuo SC, Ho TY, Hsiang CY. Ginger and its bioactive component inhibit enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea in mice. J Agric Food Chem 2007; 55(21): 8390–8397. http://dx.doi.org/10.1021/jf071460f
7. Ahmed AA, Bishr MM, El Shanawany MA, Attia EZ, Ross SA, Pare PW. Rare tri substituted sesquiterpenes daucanes from the wild *Daucus carota*. Phytochemistry 2005; 66: 1680–1684. http://dx.doi.org/10.1016/j.phytochem.2005.05.010
8. Abdulaali NI. Effect of carrot extracts on *Pseudomonas aeruginosa*. Pakistan J of Nutrition 2009; 8(4): 373-376. http://dx.doi.org/10.3923/pjn.2009.373.376
9. Casin I, Breuil J, Darchis JP, Guelpa C, Collatz E. Fluoroquinolone resistance linked to GyrA, GyrB, and ParC mutations in *Salmonella enteric* typhimurium isolates in humans. Emerging Infectious Diseases 2003; 9(11): 1455-1457. http://dx.doi.org/10.3201/eid0911.030317
10. Aboltins CA, Dowsey MM, Buising KL, Peel TN , Daffy JR, Choong PFM *et al*. Gram-negative prosthetic joint infection treated with debridement, prosthesis retention and antibiotic regimens including a fluoroquinolone. Clin Microbiol Infect 2011; 17: 862–867. http://dx.doi.org/10.1111/j.1469-0691.2010.03361.x
11. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. Drugs 2007; 67: 351–368. http://dx.doi.org/10.2165/00003495-200767030-00003
12. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA *et al*. Antimicrobial-resistant pathogens associated with healthcare-associated infections: Annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention. Infect Control Hosp Epidemiol 2008; 29: 996-1011. http://dx.doi.org/10.1086/591861
13. Adwan G, Abu Shanab B, Adwan K. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant *Pseudomonas aeruginosa* strains. Asian Pacific J of Tropical Medicine; 2010. p. 266-269.
14. El Shouny WA , Magaam S. Sensitivity of multidrug- resistant *Pseudomonas aeruginosa* isolated from surgical wound infections to essential oils and plant extracts. World J Med Sci 2009; 4(2): 104-111.
15. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—9th ed. M07-A9; Replaces M07-A8; 2012.
16. Sudhir K, Nancy, Devendra S, Vijay K. Evaluating the antibacterial activity of plant extracts against bacterial pathogens. J of Drug Delivery and Therapeutics 2012; 2(4): 182-185.

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:

Al-Alak, Shaymaa Khudhr; Ali, Wala'a Shawkat; Kadum, Maha Mikhlef and Othman, Nabaa Abd-Alkhalik. Effect of carrot and ginger extracts (alone and in combination with ciprofloxacin) on multidrug resistant Pseudomonas aeruginosa. J Pharm Sci Innov. 2014;3(2):114-116 http://dx.doi.org/10.7897/2277-4572.032121