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RESEARCH ARTICLE

Antibacterial Effect of Hot Peppers (*Capsicum annuum*, *Capsicum annuum var globriusculum*, *Capsicum frutescens*) on Some *Arcobacter*, *Campylobacter* and *Helicobacter* Species

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ABSTRACT

In this study, water, ether and methanol extracts of the hot pepper (*Capsicum annuum*, *Capsicum annuum* var *globriusculum*, and *Capsicum frutescens*) were used as antimicrobial compounds. The pepper was shredded into small particles using a sterile scalpel and dried in a dark environment at room temperature. Dried peppers were grinded and 150 g of powder was separately thawed in 150 ml of water, 150 ml of methanol and 150 ml of ether. Samples were left for 24 hours for maceration. Samples were filtered through the Whatman No.1 filter paper. The liquid portion was evaporated to 1 ml thick with vacuum evaporator to obtain stock of extracts (25^{o}C) (temperature is correct). The microdilution method was used to determine the antimicrobial activities of the extracts. The stock and serially diluted (2 folds) plant extracts were tested on *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii*, *Helicabacter pylori* and *Campylobacter jejuni*, which were prepared as standardized inoculum (1-2 x 10^6 CFU/ml). The results were measured spectrophotometrically at 405 nm wavelength. The *in vitro* activity of hot pepper extracts was determined at different concentrations on the bacteria tested. It was determined that the methanol extract of *Capsicum annuum* (*C. annuum*) was effective on *Helicabacter pylori* and *Campylobacter jejuni,* while its water extract was highly effective on *Arcobacter cryaerophilus*.

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INTRODUCTION

Hot peppers are plants that are prevalently grown in countries like Turkey, China and Mexico. Various pepper species, mainly *C. annuum* are added to several types of products and consumed as food (Yentür *et al*., 2012). *C. annuum* belongs to the *Solanaceae* family and contains 20 capsacinoids such as nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin and nonivamide (Santos *et al*., 2012; Barbero *et al*., 2014).

Capsaicin (Trans-8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide) form 79-90% of these capsaicinoids. Capsaicinoids reach their maximum concentration in peppers (1789 µmol/kg) during the developmental period. Water insufficiency, infections and mineral substance addition increase capsaicinoid levels while reducing the maturation rate and the activity of the peroxidase enzyme (32%). The peroxidase enzyme oxidizes both capsaicin and dihydrocapsaicin, which have been demonstrated in *in vitro* experiments (Santos *et al*., 2012; Barbero *et al*., 2014). Capsaicinoids are soluble in water, ethanol, methanol, acetone, acetonitrile and ethylacetate. Capsaicin and dihydrocapsaicin could be identified in the ethylacetate extract from *C. annuum* chili (Koffi-Nevry *et al*., 2012). An average of 8-16 mg/g capsaicin and 0.65-9.17 mg/g dihydrocapsaicin were detected in the extracts prepared with acetone, acetonitrile and ethanol, taken from the seeds, stems and bark of the kiln-dried chili pepper. *C. annuum* has been shown to contain flavonoids, alkaloids (vaniloids), polyphenols and steroids as well as capsaicinoids (Chinn *et. al.*, 2011). Furthermore, proteins called "defensin" which have antibacterial, antifungal, antiviral, antitumor and antiparasitic characteristics have been identified in peppers (*C. annuum*) (Barbero *et al*., 2014; Argaez *et al*., 2016; Guillen-Chable *et al*., 2017; Santos *et al.*, 2017). The industrial effects of hot peppers are caused by their contents of capsaicinoids, polyphenols and defensins.

Water and methanol extracts prepared from *C. annuum* and *C. frutescens* were shown to be effective against *Bacillus cereus, Bacillus subtilis, Clostridium sporogenes, Clostridium tetani, E. coli, Enterococcus faecalis, Salmonella Typhimurium, Staphylococcus aureus, Streptococcus mutans* and *Vibrio cholerae* (Kuda *et al*., 2004; Santos *et al*., 2012; Koffi-Nevry *et al*., 2012; Silva *et al*., 2013; Costinescu *et al*., 2015). The defensins obtained from *C. annuum* have fungicidal effects as well as the effects on bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Defensins that bind to phospholipids impair the permeability of cell walls and cell membranes. They inhibit enzyme and protein synthesis, activate ion channels, and have cytotoxic effects (Guillen-Chable *et al*., 2017). *C. annuum* L has 8 different fungistatic proteins in its fruit extracts (Santos *et al*., 2017). Proteins that inhibit the growth of cancer cells have been obtained from *Capsicum chinense*. It is suggested that the antitumor effects of these proteins are due to their reducing effects on the toxicity caused by radiotherapy and antiviral drugs. These proteins have also been shown to be effective on microorganisms such as *Xanthomonas campestris, Erwinia carotovora, P. syringae, P. aeruginosa, Shigella flexnerii, Staphylococcus aureus* and *Saccharomyces cerevisiae* (Argaez *et al*., 2016).

Fresh peppers (*C. annuum*) contain Vitamin C, E, Provitamin A, carotenoid and phenolic compounds, which have antioxidant effects (Silva *et al*., 2013). Alcoholic extracts from fresh *C. annuum* have been shown to exhibit antioxidant properties (Silva *et al*., 2013; Barbero *et al.*, 2014; Costinescu *et al*., 2015).

Peppers are used in cheese and raw milk for antioxidants and antibacterial purposes (Sricharoen *et al*., 2017), and chili peppers are used in food, cosmetics and clothing dyes (Chinn *et al*., 2011). Peppers are reported to have antidiabetic and anilipidemic effects (Kuda *et al*., 2004; Sricharoen *et al.*, 2017). Alcoholic extracts derived from pepper have the potential to be used as antirheumatic, antiinflammatory and antimicrobial agents (Costinescu *et al*., 2015). It is suggested that some of the compounds contained in the peppers may be used in some cancers, gastric ulcers, immunodepression, cataracts, macular degeneration and cardiovascular diseases (Silva *et al*., 2013; Barbero *et al*., 2014). Studies have shown that bioactive textile materials with antimicrobial effect can be developed from peppers (Ksiba *et al*., 2015).

Among the side effects, excessive exposure to capsaicin has been reported to cause local irritation, respiratory cancers and respiratory problems (Chinn *et al*., 2011). There are large numbers of bacteria that cause infections in humans and animals. These include *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii* (Aerobic bacteria) *Campylobacter jejuni* and *Helicobacter pylori*. These bacteria are Gram (-) and microaerophilic. They cause gastroenteritis in humans and animals. Stomach ulcers particularly caused by *H. pylori* are seen frequently in humans, cats and dogs. Treatment of this type of stomach ulcers frequently involves metronidazole, omeprazole and clarithromycin (Doğan, 2017). This is why it is highly important to prevent resistance development and use inexpensive medicines with low side effects.

It is known that very important medicines can be discovered by taking advantage of ethnopharmacology. It is known that people in Kars-Erzurum region (Turkey) eat peppers to relieve gastric pain. Determination of antimicrobial effects of *C. annuum*, *C. annuum* var. *globriusculum* and *Capsicum frutencens* on bacteria can leads up their use in gastrointestinal infections and the synthesis of new drugs.

The aim of this study was to investigate the effects of *Capsicum annuuum, C. annuum* var *globriusculum* and *C. frutescens* on *Arcobacter cryaerophilus, Arcobacter skirrowii, Arcobacter butzleri, Campylobacter jejuni* and *Helicobacter pylori*.

MATERIALS AND METHODS

In this study, we used long hot pepper (*C. annuum*), hot bell pepper (*C. annuum* var globriusculum) and conical ball hot pepper (*C. frutescens*), and laboratory isolate strains of *Arcobacter cryaerophilus, Arcobacter skirrowii, Arcobacter butzleri, Campylobacter jejuni and Helicobacter pylori*. Peppers were identified botanically in the Department of Biology at the Faculty of Arts and Sciences, Kafkas University. The peppers were dried at room temperature and in the dark and fragmented in a mixer. In Erlenmeyer flasks, 150 g of each pepper type was placed and 150 ml of solvent was added. It was thoroughly mixed and then allowed to maceration for 24 hours. The samples were filtered through a Whatman No.1 filter paper and then the filtrate was evaporated at 25°C in evaporator until the volume reached 1 ml. (Özkazanç, 1979). In this way, three different extracts, including water, methanol and ether were prepared from each pepper sample (a total of 9 extracts in the study). Extracts were stored at room temperature and in darkness (Maximum 5 days). A modified version of the Broth Dilution method reported by Erıcsson and Sherries (1971) was used to determine the antibacterial effect (Minimum inhibitory concentration). In the antibacterial test, stock (1/1) and serially diluted solutions of extracts were used (1/2, 1/4, 1/8, etc.). Mueller-Hinton Broth (Becton Dickinson, 211443) was used for bacterial dilution and also for breeding.

A 24-hour fresh bacterial culture was prepared on solid culture medium and then bacterial concentrations adjusted according to McFarland 0.5 standard tube and the final bacterial concentrations were used after being diluted 100 times. An equal amount of bacterial suspension was added onto extracts serially diluted with Stock and Mueller-Hinton Broth (standardized strains of *Arcobacter cryaerophilus, Arcobacter skirrowii, Arcobacter butzleri, Campylobacter jejuni* and *Helicobacter pylori* at the rate of 1×10^6 CFU/ml were used). Tests were run in duplicate and different positive and negative control tubes were prepared for each solution. The media were allowed to incubate at 37°C for 24 hours. The results were evaluated on a spectrometer at a wavelength of 405 nm. The statistical comparison of the groups was carried out by one-way analysis of variance (ANOVA) using Duncan's method. The tests were conducted by utilizing the SPSS 20 software.

RESULTS AND DISCUSSION

The results of the study showed that extracts prepared from peppers were effective at varying concentrations on the tested bacteria. Stock solutions of *C. annuum, C. annuum* var globriusculum and *C. frutescens* prepared by using water, methanol and ether and diluted solutions prepared from these stock solutions were found to be effective at variable concentrations (depending on the bacteria and diluted solution) on *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii, Campylobacter jejuni* and *Helicobacter pylori*. The results obtained from the study are summarized in Table 1. There was no significant difference among the groups (P>0.05).

Today, several medicines are being used against the infections caused in humans and animals by *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii* (Aerobic bacteria) *Campylobacter jejuni* and *Helicobacter pylori*. Metronidazole, omeprazole and clarithromycin are drugs that are frequently used especially in treatment of stomach ulcers caused by *H. pylori* (Doğan, 2017). This promotes the emergence of resistance in bacteria against antibacterial medicines. This is why it is greatly important that selective and harmless new antimicrobial drugs are developed for usage in treatment of and protection from (including food preservatives) such diseases. It is seen in the literature that various plants such as hot peppers have been studied for this purpose **(**Irkin *et al*., 2010).

The methanol and water extracts from *C. annuum* were found to have a minimum inhibitory concentration level of 0.20 mg/mL for *Vibrio cholerae, Staphylococcus aureus* and *Salmonella Typhimurium*. However, the methanol and water extracts obtained from *C. frutescens* were found to have a minimum inhibitory concentration level of 0.25 mg/mL, whereas the minimum bactericidal concentrations of methanol and water extracts from both pepper species were found to be 1-2.5 mg/mL for the same bacteria (Koffi-Nevry *et al*., 2012). It has been shown that crude extracts obtained from seeds of *C. frutescens* have an inhibitory effect (Inhibition zone ≥ 13 mm) against *Pesudomaonas aeruginosa, Klebsilla pneumoniae, Staphylococcus aureus* and *Candida albicans* (Gurnani *et al*., 2016). The number of bifidobacteria in the serum of mice fed with red pepper (*C. annuum* var conoides 2%, in a diet containing 19% fish oil) and garlic for 4 weeks decreased from 9.4 to 9.0 log CFU/g, and the number of Staphylococci from 8.7 to 7.6 log CFU/g. The same study also showed a decrease in plasma triglyceride levels (Kuda *et al*., 2004). *C. annuum* extract was added to the minced beef and stored at 7°C for 7 days and then its effect on *Salmonella Typhimurium* and *Pseudomonas aeruginosa* was investigated. The minimum inhibitory concentration on *S. Typhimurium* was determined as 1.5 ml/100 g meat. No additional inhibitor effect on *S. Typhimurium* was observed for sodium chloride added at the concentrations of 1, 2, 3 and 4% along with the extract. The minimum inhibitory concentration of the extract was determined to be 3 ml/100 g for *P. aeruginosa*. Sodium chloride added to the mince at a concentration of 1% along with the extract was found to significantly reduce the minimum inhibitory concentration level of the extract for *P. aeruginosa* (Careaga *et al*., 2003). Investigation of the effects of aqueous extracts obtained from *C. annuum*, *Capsicum baccatum*, *Capsicum chinense*, *C. frutescens* and *Capsicum pubescens* on fifteen bacterial and yeast species by disk diffusion method revealed that the pepper extracts exhibit varying degrees of inhibition against *Bacillus cereus, Bacillus subtilis, Clostridium sporogenes, Clostridium tetani* and *Streptococcus pyogenes* (Cichewicz and Thorpe, 1996).

The antibacterial effects of pepper extracts can be suggested to originate from the compounds determined in the pepper. The minimum inhibitory concentration values of capsaicin and dihydrocapsaicin isolated from the ethyl acetate extract of fruits of *C. annuum* chili, and synthetic derivatives including (N-(4-hydroxyphenylethyl) decamide, (E)-N-(4-hydroxy-3-methoxybenzyl)-3,7-dimethylocta-2,6-dienamide, 4-hydroxy-3-methoxy-N-((E) -3,7-dimethyloctane-2,6-dienyl) benzamide and N-(4-hydroxy-3-methoxybenzyl) decamide for *Sterptococcus mutans* were 2.5 mg / mL, 1.25 µg/mL, 5.0 µg/mL and 2.5 µg/mL for the last three, respectively (Sntos, *et al*., 2012). The defensins in the pepper extracts are also responsible for their antibacterial effects (Guillen-Chable *et al*., 2017). The peptides isolated from seed extracts of *Capsicum chinense* were found to be effective on *Xanthomonas campestris, Erwinia carotovora, P. syringae, P. aeruginosa, Shigella flexnerii* and *Staphylococcus aureus*. They have also been shown to inhibit the growth of *Saccharomyces cerevisiae* and Hep-G2, PC-3 and Si-Ha cancer cells (Argaez *et al*., 2016).

In this study, stock solutions of *C. annuum, C. annuum* var globriusculum and *C. frutescens* prepared by using water, methanol and ether and diluted solutions prepared from these stock solutions (1/1) were found to be effective at variable concentrations (depending on the bacteria and diluted solution) on *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii, Campylobacter jejuni* and *Helicobacter pylori.* These results are consistent with the results of other studies showing that pepper extracts have antibacterial effects.

Table 1: The concentrations of *C. annuum*, *C. annuum* var *globriusculum*, *C. frutescens* extracts that are effective on bacteria

Bacterium	EC of *C. annuum*			EC of *C. annuum* v. globrius.			EC of *C. frutescens*		
	WE	ME	EE	WE	ME	E	WE	ME	E
Arcobacter butzleri	NE	NE	1/2	1/1	NE	1/1	1/2	1/1	1/1
Arcobacter cryaerophilus	1/32	1/2	1/2	NE	NE	1/2	NE	1/1	1/1
Arcobacter skirrowii	NE	1/1	1/1	NE	NE	1/1	1/2	1/1	1/1
Helicobacter pylori	NE	1/32	NE	NE	NE	1/1	NE	NE	1/1
Campylobacter jejuni	NE	1/16	1/2	NE	NE	1/2	NE	NE	1/1

EC: Effective concentration, WE: Water extract, ME: Methanol extract, EE: Ether extract, NE: No effect.

It is known that various experiment studies have been carried out for the use of peppers in treatments. The effects of diet supplemented with red pepper and turmeric (Oleoresin) on (NE) necrotic enteritis model in chickens induced by *Eimeria maxima* and *Clostridium perfringens* have been investigated. The numbers of *Eimeria maxima, C. perfiringens* and *Aerobacter* and necrotic enteritis induced weight loss and intestinal lesions were significantly reduced due to the pepper and turmeric supplied with the water (Kim *et al*., 2015). Some studies support the idea that pepper extracts can be added for bio-conservative purposes because of their antibacterial effects. It has been shown that *C. annuum* extract, which was added at a concentration of 5% into milk having a pH value of 4.5 with experimentally inoculated *Listeria monocytogenes*, prevented bacterial growth during 4 to 8 days of storage (Acero-Ortega *et al*., 2005). In the samples (0.5 kg) taken from the cheese (Botanero cheese) produced by the addition of peppers by families in Northeastern Mexico, the levels of Lactobacillus, Coliform (100 CFU/g-10.000 CFU/g), *S. aureus* (1,000 CFU/g-100 CFU/g) and yeast (500 CFU/g) were found to be decreased when compared to samples without pepper (Escobar-lopez *et al*., 2017). In a study by Dannenberg *et al*. (2017) cellulose acetate based active packaging films obtained from pink pepper (Essential oils, EO) were added to solid media at a concentration of 4.6% and their activity against *Listeria monocytogenes*, *Escherichia coli* and *Salmonella Typhimurium* and *Staphylococcus aureus* was investigated by diffusion technique. This study concludes that they can be used as packaging materials, depending on food penetration rates and antibacterial effects. The antibiotic and antioxidant effects of composite films prepared from red pepper for clothing and containing 0.5% pepper powder could be shown in studies. Fatty tuna meat wrapped in a composite film containing 0.5% red pepper powder was stored for 12 days at 4°C and the number of *Listeria monocytogenes* (0.73 log CFU/ml) and *Salmonella Typhimurium* (1 log CFU/ml) and lipid peroxidation levels were found to be decreased compared to controls (Lee *et al*., 2016).

Several studies have shown that peppers have antioxidant effects. Alcoholic extracts from *C. annuum* were found to have significant antioxidant effects (723.795 mg / L GAE, equivalent to 3.315 mg/L of trolox and 327.394 mg / L QE) as well as antibacterial effects (Costinescu *et al*., 2015). In n-hexane and chloroform extracts obtained from *C. frutescens* seeds, compounds such as diketones, hydrocarbons, long straight chain carboxylic acids, ester, hydroxyester, aromatic compounds, phenol and flavonoid have been identified, and it was shown *in vitro* that the free radical scavenging effects of n-hexane and chloroform extracts at 1 mg / L concentrations are 36.1% and 30.5%, respectively (Gurnani *et al*., 2016). High levels of 1-ascorbic acid in the peppers are also implicated in antioxidant effects (Garcia-Pineda *et al*., 2004). It can be suggested that antibacterial and antioxidant effects of peppers originate from capsicinoids and other chemical substances contained.

Conclusions: Water, methanol and ether extracts obtained from pepper were found to inhibit the production of microorganisms used in the experiment. The highest effects were seen on *Arcobacter butzleri* by the ether extract of *C. annuum* and ½ concentration the water extract of *C. frutescens*, on *Arcobacter cryaerophilus* by 1/32 concentration of the water extract of *C. annuum*, on *Arcobacter skirrowii* by ½ concentration of the water extract of *C. frutescens*, on *Helicobacter pylori* by 1/32 concentration of the methanol extract of *C. annuum* and on *Campylobacter jejuni* by 1/16 concentration of the methanol extract of *C. annuum*. In order to use pepper extracts for the synthesis of new drugs and for the treatment of gastrointestinal disorders, the results of our research should be supported by more extensive, *in vitro* and *in vivo*, pharmacokinetic and pharmacodynamic studies.

Authors contribution: ANCD planned to work, he interpreted the data. EÇ has made antibacterial studies. PAK prepared the plant extracts. EA interpreted the data planned to work. AGS has done antibacterial studies. AD prepared the plant extracts, he took the latest version of the article. SO evaluated the data. All authors contributed to article writing and interpreted the data.

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