

Short Communication

Investigation of antibacterial effects of garlic (*Allium sativum*), mint (*Menthe spp.*) and onion (*Allium cepa*) herbal extracts on *Escherichia coli* isolated from broiler chickens

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This study was done to determine minimum inhibitory concentration (MIC) of the aqueous extracts of garlic (*Allium sativum*), mint (*Menthe spp.*) and onion (*Allium cepa*) in *in vitro* conditions against the *Escherichia coli* isolated from broiler chickens. *E. coli* was isolated from the infected tissues of the chickens which were suspected of *Colibacillus* infection. In this study, distilled water, phenol phenicol and floxacillin antibiotics were used as control. *E. coli* was sensitive to antibiotics, but distilled water had no inhibitory effect on the activity of *E. coli*. In this experiment, each of the aqueous extracts was prepared by using distilled water in 6 concentrations: 0.5, 1, 2.5, 5, 10 and 20%, and was poured into the cavities in nutrient agar medium, and then the plates were kept in incubator at 37°C for 24 h. The results indicated that MIC of the garlic aqueous extract was 5%, but *E. coli* was resistant to the aqueous extracts of onion and mint.

Key words: Herbal extracts, garlic, onion, mint, *Escherichia coli*, broiler chickens, minimum inhibitory concentration, antibacterial effects

INTRODUCTION

Pharmaceutical plants have been commonly used by humans long ago and the consumption rate has changed based on the occasion and the requirement of the time. In recent years, Iranians and other people from around the world have shown a tremendous interest in these plants as a way to cure their illnesses. Today, there have been several attempts to develop these plants and the derivatives because the ever-increasing usage of the chemical drugs has led to serious problems and created resistant types of insensitive micro-organisms, while herbal drugs bring about fewer side effects because the biological balance. The herbal extracts are either used

alone as the drug itself or constitute a part of the drug.

Escherichia coli is one of the first intestinal bacilli which has been cultured and is the intestinal flora in humans and animals. It is a facultative anaerobic bacterium that grows on certain mediums especially eosin methylene blue agar (EMBA) with metallic sheen colonies. This organism grows at the range of 10 to 46°C but optimal temperature is 37°C. Most of the strains of *E. coli* are killed at 60°C in 30 s. But some resistant strains are available. This organism, in different conditions, can act as a pathogenic bacterium, especially in secondary infections.

Most essential oils consist of mixtures of compounds such as phenolics and polyphenols, terpenoids, saponins, quinines, esters, flavones, flavonoids, tannins, alkaloids and nonvolatile residues; and their chemical composition and concentration of compounds is variable. These components have many effects as antimicrobial, stimulating animal digestive systems, antioxidants,

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Abbreviations: EMBA, Eosin methylene blue agar; MIC, minimum inhibitory concentration; GIA, growth inhibiting agent.

Table 1. The mean diameter (mm) of zones of inhibition of herbal extracts against *E. coli* activity.

Herbal extract	Concentration of extract (%)					
	0.5	1	2.5	5	10	20
<i>Allium sativum</i>	0	0.04	0.05	0.59	1.52	3.1
<i>Menthe</i> spp.	0	0	0	0	0	0
<i>Allium cepa</i>	0	0	0	0	0	0

anticoccidial, increase production of digestive enzymes and improve utilization of digestive products by enhancing liver functions (Hernandez et al., 2009).

There have been some studies on the antifungal activity of plant extracts (Wilson et al., 1997), inhibitory effects of aqueous extracts of garlic and onion (Shams et al., 2003), antimicrobial effects of garlic, ginger and lime (Onyeagba et al., 2004), antibacterial and antifungal activity of *Senecio* (Loizze et al., 2004), antimicrobial activity of garlic and onion extracts (Elnima et al., 1983) and effects of aromatic plants essential oils, lime and garlic skin on birds intestinal bacteria (Davis et al., 1994).

Plant extracts represent a rich potential source of alternative and environmentally acceptable control agents for infectious organisms due to their antimicrobial properties. Plants possess essential oils, which could be utilized for killing microorganisms.

The aim of this study is to evaluate the antibacterial effects of 3 herbal extracts- garlic (*Allium sativum*) mint (*Menthe* spp.) and onion (*Allium cepa*) on *E. coli* *in vitro* conditions and to determine the minimum inhibitory concentration (MIC) of these herbal extracts.

MATERIALS AND METHODS

Plant materials

The leaves of mint, garlic bulbs and onion were washed separately by distilled water. The plants came from the northern regions of Iran.

The method of plants extraction

The simplest method of extraction was used in this study. Since we needed the pure extract, no solvent was used for purification of extracts. First, the leaves of mint, bulbs of garlic and onion layers were washed by distilled water. Then 1 kg of each sample was kept separately in plastic container, sealed and kept in freezer at -20°C for 48 h.

Keeping samples in freezer caused the breakage of the cells; hence cell substances were extracted more easily. They were taken out of the freezer after 48 h, and after 30 min they were defrosted; the aqueous extracts were taken with a strainer and were then cleared, filtered and poured into separate dark bottles to keep them away from sunlight (Wilson et al., 1997).

Sample from infected broiler chickens and bacteria culture

First, the chickens suspected to have *Colibacillus* infection were sampled. The method was as follows: Chickens were first suf-

focated and their carcasses were cut open for anatomy. Then, samples were prepared from the infected tissues such as liver, heart, lung and air sacs. In this method, the surface of the tissues was burned with a hot knife and samples were prepared from the internal parts with sterile forceps. After that, the samples were cultured on plates containing EMB-agar medium by streaking method. Next, the plates were kept in incubator at 37°C for 24 h. The plates were taken out after 24 h. At this time, when the *E. coli* colonies with the metallic sheen were formed on the medium, the antibiogram test was performed with the earlier mentioned herbal extracts and control antibiotics.

Preparing different concentrations of the herbal extracts

In this experiment, herbal extracts were prepared in the form of solution in distilled water for 6 concentrations: 0.5, 1, 2.5, 5, 10 and 20%, respectively.

The antibacterial test and determination of the MIC

The tested bacterium was *E. coli*. At first, 9 cavities with 5 mm diameter were prepared in the plates containing nutrient agar medium by sterile drill (6 cavities for the herbal extracts, one cavity for distilled water and the two other cavities for phenol phenicol and floxacilin as control). Then, 5 to 6 metallic sheen colonies on EMB-agar medium were picked up with sterile swab and spread well on the nutrient agar medium having cavities. Next, 5 ml of all 6 concentrations for aqueous extracts (garlic, onion and mint) were poured into the cavities with a sampler and were kept in incubator at 37°C for 24 h. Finally, the plates were taken out and the zones of inhibition were measured by scale. Phenol, phenicol and floxacilin were used as control. This test was repeated 50 times.

RESULTS AND DISCUSSION

E. coli was sensitive to all the mentioned antibiotics, but the distilled water does not have any inhibitory effect on *E. coli*. Based on the results shown in Table 1, since the 1 and 2.5% concentrations of the garlic extract have minor inhibition effects (0.04 and 0.05), the MIC for the garlic extract was 5% which may be due to its active ingredients such as alin and alicin. But the mint and onion extracts showed no inhibitory effects against the *E. coli*, which may be related to the method of extraction. According to the results obtained, garlic aqueous extract can be considered as a growth inhibiting agent (GIA) against *E. coli*.

The results of this experiment are similar to reports provided by Zennere et al. (2003), which stated that minimum bactericidal concentration of the garlic essential

oil on intestinal bacteria of poultry was 0.125 ml; Elnima et al. (1983), which maintained that the garlic extract when compared to onion extract, has more effects on the oral bacteria; Sreter et al. (1999), which remarked that the garlic extract in comparison with the anti-coccidiosis drugs used for the chickens was more effective; Kumar and Bormal (1998), which explained that the 80% concentration for garlic had inhibition effects on the *E. coli*, *Salmonella* and *Staphylococcus*; Davis et al. (1994), which showed that the 6 to 12 mg/ml concentration of garlic had antifungal activity; Wilson et al. (1999), which stated that out of 345 extracts under the study for antifungal activity, the activity of garlic extract was more; and Shams et al. (2003) which reported that the 10% concentration of aqueous extracts of garlic and onion had antifungal activity.

Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents. Understanding of plant biochemistry, physiology and chemistry of natural products have shown that the secondary metabolites may be used to control infectious organisms to overcome the earlier mentioned problems associated with synthetic chemicals (Delaquis and Mazza, 2008). As a result, increased interest is being shown in developing alternative methods for microbial contamination control to reduce or eliminate reliance on synthetic pesticides. One of such method involves the use of plant-derived-products such as plant essential oils that has antimicrobial effect.

However, the findings of this research do not have anything in common with those of Onyeagba et al. (2004) who remarked that when the aqueous extracts of garlic and ginger were used alone, they did not have any inhibition effect on *E. coli*, *Salmonella* and *Staphylococcus*, but when used with the lime extract, showed inhibitory effect.

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