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ORIGINAL ARTICLE

Evaluation of Antimicrobial activity of *Eucalyptus* essential oil and *Urtica* alcoholic Extract on *Salmonella enteritidis* and *Shigella dysenteriae in Vitro* condition

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ABSTRACT
Nowadays, medicinal plants have many applications in people's lives. They can be used in the pharmaceutical compounds, cosmetic, sanitary and nutritional industries. Antiviral, antibacterial and antifungal activity of the plants has a long history. In this study we examined the antimicrobial activity of Eucalyptus essential oil and Urtica alcoholic extract on Salmonella enteritidis and Shigella dysenteriae In Vitro condition. The plant was collected in summer 2014 from Lashkarak hill located in eastern north of Tehran. Eucalyptus essential oil and Urtica alcoholic extract were tested separately against two species of bacteria. Data were analyzed by ANOVA using a SAS system procedure. Our data revealed that plant Eucalyptus essential oil and Urtica alcoholic extract maybe have inhibitory effect on Salmonella enteritidis and Shigella dysenteriae. We also found that Urtica alcoholic extract was more effective compared to the Eucalyptus essential oil.
Keywords: Antibacterial activity, Urtica

INTRODUCTION

Medical herb is a branch of traditional medicine of countries such as Iran, which had been played vital role in treatment of disease till a century ago [1]. Antiviral, antibacterial and antifungal activity of the plants has a long history. After the discovery of Penicillin, as one of the most important life-saving phytodrugs, scientists has refocused on plant origin, antimicrobial and antidermatophytic agents. After that, several studies have tested many extracts from different plants for their antimicrobial activities but there are so many unopened pages of plant life which require more careful investigation to reveal their hidden characteristics. Generally natural products are considering being harmless or having minimum side effects as compared to synthetic drugs or have minimum side effects as compared to synthetic drugs. Several antimicrobial agents were isolated from plant including secondary metabolites as essential oil and Terpenoides, amongst them xanthones, benzophenones, coumarins and flavonoids can be mentioned [2]. The Enterobacteriaceae are a large family of Gram-negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*. Eucalyptus is a tall, evergreen tree, native to Australia and Tasmania, and is successfully introduced worldwide. It is now extensively cultivating in many other countries including Iran. Its therapeutic uses have been introduced and integrated into traditional medicine systems. Eucalyptus species extracts are now entering into common herbal use for the treatment of cold, chest pain, or cough. Eucalyptus leaf extracts have been used to treat influenza, chest problems, and skin rashes while their vapour is inhaled to fight inflammation [3]. *Urticadioica* is known as a common herb worldwide. Its herapeutic use has been reported for rheumatic pain, urinary tract infections and bladder stone [4]. It has several pharmacological properties such as anti- inflammatory, antimicrobial, ant oxidative activities and hepatoprotective and cardioprotrctive effects [5, 6]. Several studies have shown that *Urticadioica is able to* decrease the lipid peroxidation and liver enzymes, and to increase the antioxidant defense system activity in the carbon tetrachloride- treated rats [7, 8].

MATERIALS AND METHODS

In this interventional study, the plant was collected in summer 2014 from Lashkarak hill located in eastern north of Tehran. Taxonomy of plant materials was verified by data of the biological part of medical college of Azad University of Tehran and assisting from the book of Dr. Vali-allahMozafarian. Collected plat materials were dried under the shadow and its leaves removed from the stem and then they were powdered separately. Powdered samples of stem and leaves were extracted by floating in methanol and then by hot water. Nearly 200 gr of powder for each sample (leaves and stems) solved in 600 ml of methanol and then left for about 48 h in the ambient temperature (25°) while shaking slowly. Each extract filtered by Watman paper filter No.1. Filtered extracts were dried in the room temperature by air flow [9, 10, and 11]. Extracts dried by UV ray were sterilized at night and their sterility was studied on nutrient agar medium by culturing the plant extract. All dried extracts stored in room temperature until examination. Later, *Eucalyptus* essential oil and *Urtica* alcoholic extract were tested separately against two species of bacteria. Microorganisms were provided in the microbiology section of medical college of Islamic Azad University of Tehran. The identity of test microorganisms was verified by standard microbial identification system of this sector.

For MIC and MBC determinations, serial twofold dilutions of *Eucalyptus* essential oil and *Urtica* alcoholic extract in 5 ml of broth inoculated with 50 µl of fresh precultures (inoculum, ~108 CFU/ml). The tubes were incubated at 37°C overnight with shaking and the highest dilution in which there were no growth was recorded as the MIC. For MBC testing, aliquots (20 µl) of broth from tubes containing no growth were plated onto solid medium and again incubated overnight at 37°C. The highest dilution in which there were no survivors was recorded as the MBC. In the above method, controls for each organism were performed using the sterile liquid medium without aqueous garlic extract. All MICs and MBCs were confirmed by triplicate assays. Data were analyzed by ANOVA using a SAS system procedure (SAS Institute, Cary, NC, USA). A multiple comparison test (least significant difference) was used to test for significant differences between the treatment means ($P< 0.05$).

RESULTS

Results indicated that *Eucalyptus* essential oil and *Urtica* alcoholic extract inhibited the growth and killed *Salmonella enteritidis* and *Shigelladysenteriae*. In order to study the effect of the E.E.O. and U.A.O., different concentrations were tested. No effect on the different cell growth curves was observed when we added an E.E.O. and U.A.O. to a final concentration less than 11 mg/ml. So, we had studied the effect of *Eucalyptus* essential oil and *Urtica* alcoholic extract added to a diluted final concentration, which range from 11 mg/ml to 13 mg/ml. Compared with the control cell suspensions without *Eucalyptus* essential oil and *Urtica* alcoholic extract, we observed a modification of the classical cell growth curves of the different *Salmonella* and *Shigella*serovars. The addition of E.E.O. and U.A.O.to these final concentrations at cell density of 0.05 induces the apparition of an inhibition phase in all the *Salmonella* and *Shigella* growth curves.

After this inhibition phase, cell growth resumed but at rate inferior to the control cell suspensions. In addition, cultures exposed to E.E.O. and U.A.O. entered stationary phase at a cell density substantially lower than that of the control culture. This phenomenon was observed in all cases of E.E.O. and U.A.O. treated*Salmonella* and *Shigella*. As the *Eucalyptus* essential oil and *Urtica* alcoholic extract concentration was increased from 11 to 13 mg/ml the duration of the inhibition phase increased, the rate of the growth after inhibition decreased, and the cell density at which stationary phase was entered also decreased. The duration of the inhibition phase was proportional to the *Eucalyptus* essential oil and *Urtica* alcoholic extract concentration and variable according to the different *Salmonella* and *Shigella* tested. A longer growth inhabitation phase was observed using increased E.E.O. and U.A.O. concentration (Table 1 and 2).

Table2: Effect of U.A.O. concentration on the different *Salmonella enteritidis* and *Shigelladysenteriae* cell growth curves.

		11 mg/mL	12 mg/mL	13 mg/mL
Duration of inhibition (min)	*Salmonella enteritidis*	120 ± 0.71	360 ± 0.71	720 ± 1.41
	Shigella dysenteriae	360 ± 0.71	540 ± 0.00	600 ± 0.71
Resumed growth rate (% of uninhibited rate)	*Salmonella enteritidis*	100 ± 1.41	81 ± 0.71	2.5 ± 0.71
	Shigella dysenteriae	51 ± 1.41	44 ± 0.00	32 ± 0.00

Table3: Effect of E.E.O. concentration on the different *Salmonella enteritidis* and *Shigelladysenteriae* cell growth curves.

		11 mg/mL	12 mg/mL	13 mg/mL
Duration of inhibition (min)	*Salmonella enteritidis*	240 ± 1.41	364 ± 0.71	480 ± 1.41
	Shigella dysenteriae	480 ± 0.00	480 ± 1.41	600 ± 0.00
Resumed growth rate (% of uninhibited rate)	*Salmonella enteritidis*	74 ± 1.41	69 ± 0.71	46 ± 0.71
	Shigella dysenteriae	87 ± 0.71	77 ±0.00	63 ± 0.71

DISCUSSION

With the emergence of antibiotic resistant bacteria, it is reasonable to search new sources of natural compounds with antimicrobial activity. Edible plants have been proven to be harmless and economical. Base on the results, *Eucalyptus* essential oil in this study have significant antimicrobial activity on the studied microorganisms. Antimicrobial effect of the extracts was different, depending on the type of microorganisms. Also, *Urtica* alcoholic extract compared to the *Eucalyptus* essential oil was more effective and has a greater deterrent. The reason of these phenomena may be extracting more effective materials extracted. Other studies indicated that Urtica didn't have any effect on the *Bacillus subtilis*, *Bacillus cereus* and *Bacillus pusilus* but it affected on the *Pseudomonas aeruginosa* and *pseudomonas fluorescence*. In other words, Inhibition zone diameter was 10 mm for both of them but MIC was 20 and 25 for *P. aeruginosa* and *fluorescence* correspondingly [14]. These results are consistent with the findings of a study by Mahasneh [12] on Qataris mangrove species and it is found the aqueous mangrove extract, don't have a significant antimicrobial effect, and the butanol extract, is able to inhibit *Pseudomonas aeruginosa*. Sattari *et al.* [13] showed 3.2 µg / ml concentration of alcoholic *Eucalyptus* extract and 17.5 µg / ml concentration of aqueous *Eucalyptus* extract, can be good to prevent the growth of *Pseudomonas aeruginosa* standard isolates. Our data revealed that plant *Eucalyptus* essential oil and *Urtica* alcoholic extract maybe have inhibitory effect on *Salmonella enteritidis* and *Shigella dysenteriae* and *Urtica* alcoholic extract compared to the *Eucalyptus* essential oil was more effective.

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