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Antimicrobial Effect of Several Oil Extracts on *Escherichia coli*

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Abstract: This study investigated the antimicrobial effect of different oil extracts on *Escherichia coli* bacterium isolated from raw milk. The isolated strains of *E. coli* were tested for their resistance to ten oils extract using disk diffusion method. The oils used were extracted from Cinnamon, Black pepper, Black seed, Garlic, Onion, Hibiscus, Sage, Aniseeds, Thyme and Mint. The oil extracts were dissolved in dimethoxy sulfoxide (DMSO) in concentrations of 20, 40 , 60 and 80%. The results showed that Cinnamon, Black pepper, Black seed, Garlic, Onion, Hibiscus, Sage and Thyme oil extracts have inhabited the growth of *Escherichia coli* at all concentrations used. However, Aniseeds and Mint oil extracts were found to be ineffective against the isolated bacteria regardless of the concentration used.

Key words: Oil extracts · *Escherichia coli* · Cinnamon · Black pepper · Black seed · Garlic · Onion · Hibiscus · Sage · Aniseeds · Thyme · Mint

INTRODUCTION

Food shelf life is a vital topic to consumers, producers, regulators and researchers as well. There have been increasing consumer concerns about food free or with lower levels of chemical preservatives because of their possible poisonous effect on humans [1]. Recently, there has been increasing interest in discovering new natural antimicrobials. Plant products with antimicrobial properties have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth [2]. There has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. Those are, in the first place, extracts and essential oils (EOs) of spices and herbs [3]. As natural foodstuffs, spices and herbs appeal to all who question safety of synthetic food additives and demand high-quality products that at the same time are safe and stable [4]. Spices and herbs have been added to food since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives [5-7].

Escherichia coli (*E. coli)* is one of the most common bacteria causing food borne diseases worldwide [8]. It is a gram-negative rod (bacillus) bacterium in the family Enterobacteriaceae. Most of *E. coli* is normal commensals found in the intestinal tract. *E. coli* is an important member of the normal intestinal microflora of humans and other mammals; it has also been widely exploited as a cloning host in recombinant DNA technology. But *E. coli* is more than just a laboratory workhorse or harmless intestinal inhabitant; it can also be a highly versatile and frequently deadly, pathogen. Several different *E. coli* strains cause diverse intestinal and extra intestinal diseases by means of virulence factors that affect a wide range of cellular processes [9].

Various studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including foodborne pathogens [5, 10-11]. It has been reported that spices owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins [12]. Abdel-Raouf *et al.* [13] examined food poisoning bacteria in different food samples and studied antibacterial activity of ethanolic extracts of some medicinal plant. Their results showed that seven spices (Cloves, Cinnamon, Black cumin seeds, Cumin, Black and White pepper and Ginger) have an inhibitory effect against *Salmonella*, *S. aureus* and *B. cereus* which are important pathogens in food poisoning. Moreover, Cloves, Black cumin seeds extracts were found to be the most effective plants against almost tested microorganisms. Fennel, Garlic, Cardamom and Red chili pepper were found to be ineffective against the isolated bacteria. Rasool [14] tested the antimicrobial

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effects of rosemary (*Rosemarinus officinalis*), clove (*Sygium aromaticus*), cumin (*Cuminum cyminum*) and ginger (*Zingiber officinale*) alone and with tetracycline antibiotic against *E.coli* growth. The results emphasized that antibacterial properties of rosemary, clove and ginger essential oils in combination with tetracycline against *E. coli* were much higher than that of tetracycline; such combinations can be recommended for therapeutic purpose and be used as alternative medicine. Amrita *et al.* [15] found that many herbs and spices were able to inhibit *E. coli* growth, but Thyme (herb) and Turmeric (spice) were found to be most effective against it. Raho and Benali [16] investigated the *in vitro* antimicrobial activities of essential oil of the leaves of *Eucalyptus globulus* against *E. coli* and *S. aureus*. The results showed that the addition of the essential oil leaves in broth culture inoculated with *E. coli* and *S. aureus* inhabited the growth of these organisms. The rate of inhibition was greater on gram negative bacteria *E.coli* than that observed on gram positive bacterium *S. aureus*. El-Kholie *et al.* [17] studied the effect of spices (cinnamon, cloves and ginger), different concentrations (0, 0.4, 0.8, 1.2 and 1.6 g/L) in powder and in oil forms (individually and as a mixture) on some pathogenic microorganisms (*E. coli*, *S. aureus*, *B. cereus*, *Salmonela sp.*, *Aspergillus niger* and *Candida albicans*) in liquid media. The results showed that the highest inhibition percentage was recorded with spices mixture powder when compared to the other tested individual spice powder. The obtained results indicated that the spices mixture oils had a higher inhibition effect than that of spices mixture powder. This study investigated the antimicrobial effect of ten oil extracts on *E. coli*.

Materials and Methods

Bacterial Isolation and Purification: A loopful from raw milk was inoculated on violet red bile broth media and incubated at 37°C for 24 hours. Then, a loopful of the culture was streaked onto violet red bile agar media then incubated at 37°C for 24 hours and examined for colony character according to Harvey and Price [18]. The pink colonies on the violet red bile agar indicate the presence of *E.coli* bacteria. After that they were streaking for five times in the same media for purification the colony. Finally, the bacteria were identified by microscopical examination according to Cruickshank *et al.* [19].

Biochemical Identification: Identification of the gram negative isolates was done according to [20-25]. The tests used in identification included: Methyl Red (MR), Voqus Proscour(VP), indole and citrate utilization tests. *E. coli* is positive indole, positive M.R , negative V.P and negative citrate according to Cruickshank *et al.* [19].

Preparation of Oil Extracts: Fresh oil extracts were bought from "Durra Food Products Company" at the same day of production. The oil extracts were dissolved in dimethoxy sulfoxide (DMSO) in concentrations of 80, 60, 40 and 20%. The solvent was also used as a negative control. The activity towards test organisms was evaluated using the diffusion plate method [26-27].

Antimicrobial Susceptibility Test: The isolated strains of *E. coli* were tested for their resistance to some oil extracts using disk diffusion method [28]; in this test, 1 ml of an overnight culture of *E. coli* were added to 20 ml of violet red bile agar medium and left to solidify in a Petri dish. Thereafter, four wells were made in each Petri dish and filled with 15 µl. of different concentrations of certain oil extract. We got ten Petri dishes each for a certain oil extract in addition to the DMSO Petri dish. The plates were incubated at 37°C for 24 h and the antimicrobial activity was evaluated by observing a clear zone of growth inhibition. The oils used were Cinnamon, Black pepper, Black seed, Garlic, Onion, Hibiscus, Sage, Aniseeds, Thyme and Mint. Duplicate of each oil extract for the isolated strains were prepared and the average reading of each oil extract under each concentration was calculated. The inhibition zones for each oil extract were determined in accordaning to Shryock and NCCLS [29]. ANOVA was used to determine the statistical significance of the inhibition zone. It was estimated through SPSS statistical package.

RESULTS AND DISCUSSION

Table 1 shows the inhibitory effect of ten oil extracts at different concentrations on *E. coli*. The results indicated the antimicrobial effect of Cinnamon, Black pepper, Black seed, Garlic, Onion, Hibiscus, Sage and Thyme oil extracts. The inhibition zone was decreased significantly (at $P \leq 0.05$) as the oils' concentration increased in most oil extracts. The largest inhibitory effect was shown under concentration of 20%. The mint and aniseeds oils did not show any effect on the examined microorganism. Black seed oil showed the highest antibacterial effect on *E. coli* with inhibition zone of (2 mm) at concentrations of 20 and 40%. Cinnamon, Black pepper and Hibiscus oils showed inhibition zone of (1.9 mm) at same concentrations. The inhibition zone of Sage oil was (1.6 mm) at concentrations of 20, 40 and 60%

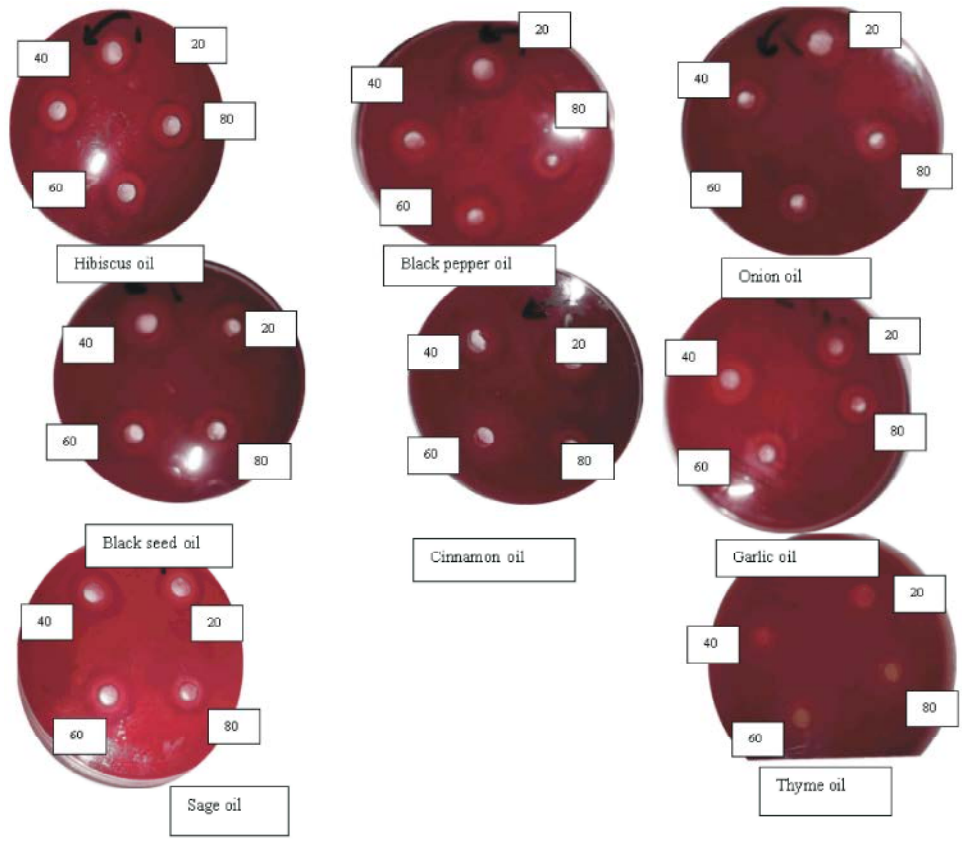

Fig. 1: The effect of several oil extracts on *E. coli.*

Table 1: Inhibition zones of oil extracts at four different concentrations

		Concentrations (%)			
		20	40	60	80
No.	Oil extracts	Inhibition zone diameter (mm)			
1	Cinnamon oil	1.9^{a}	1.9^{a}	1.8^{b}	1.5^{c}
2	Black pepper oil	1.9^{a}	1.9^{a}	1.6^{b}	1.3^{c}
3	Black seed oil	2.0^{a}	2.0^{a}	1.8^{b}	1.8^{b}
4	Garlic oil	1.6^{b}	1.8^{a}	1.4^{c}	1.2^{d}
5	Onion oil	1.5^{a}	1.3^{b}	1.3^{b}	1.3^{b}
6	Hibiscus oil	1.9^{a}	1.9^{a}	1.9^{a}	1.8^{b}
7	Sage oil	1.6^{a}	1.6^{a}	1.6^{a}	1.4^{b}
8	Aniseeds oil	0.0^{ns}	0.0^{ns}	0.0^{ns}	0.0^{ns}
9	Thyme oil	1.4^{a}	1.4^{a}	1.4^{a}	1.3^{b}
10	Mint oil	0.0^{ns}	0.0^{ns}	0.0^{ns}	0.0^{ns}

Means are averages of two replicates. Means with different letters in the same rows are significantly different at P = 0.05.

and decreased to (1.4 mm) at concentration of 80%. Garlic oil showed inhibition zone of (1.6 mm) at concentration of 20%, (1.8 mm) at concentration of 40%, (1.4 mm) at concentration of 60% and (1.2 mm) at concentration of 80%. Thyme and onion oils showed the lowest antibacterial effect on *E. coli* with inhibition zones between (1.3 mm) and (1.5 mm) at different concentrations. Aniseeds and mint oils showed no any inhibition zones indicating that these two oils cannot be used as antimicrobial agents against *E. coli.* Fig. 1 illustrates the inhibition zones for the oil extracts which showed positive results.

Amrita *et al.* [15] examined the antibacterial effect of eight herb extracts different from those which we tested and found that Thyme (herb) and Turmeric (spice) were the most effective against *E. coli*. Raho and Benali [16] showed that the essential oil of the leaves of Eucalyptus globulus has an antimicrobial effect against *E. coli*. Rasool [14] found that the antimicrobial effect of rosemary, clove and ginger essential oils in combination with tetracycline against *E.coli* were much higher than that of tetracycline alone. Zhang *et al.* [30] studied the antibacterial properties of 14 essential oil extracts (clove, oregano, rosemary, pepper, nutmeg, liquorice, turmeric, aniseed, cassia bark, fennel, prickly ash, round cardamom, dahurian angelica root and angelica) against four common meat spoilage and pathogenic bacteria (*Listeria monocytogenes, E.coli, Pseudomonas fluorescens and Lactobacillus sake*) and their results

showed that individual extracts of clove, rosemary, cassia bark and liquorice contained strong antibacterial activity, but the mixture of rosemary and liquorice extracts was the best inhibitor against all four types of microbes. Overall, spices and herb extracts are widely used in the food industry and are generally regarded as safe (GRAS). Hence, they may be considered as natural preservatives acceptable by the food industry.

CONCLUSION

Food safety is one of the most important topics for public health. Numerous researchers have investigated natural preservatives used to prevent food contamination. Essential oil extracts are ones of the most recently studied natural antimicrobial agents. In this study, we investigated the antibacterial effect of ten oil extracts against one of the most dangerous microorganism on human being, which is *E. coli.* Our results showed that Cinnamon, Black pepper, Black seed, Garlic, Onion, Hibiscus, Sage and Thyme oil extracts have inhabited the growth of *E. coli* at all concentrations used. Nevertheless, the results indicated that Aniseeds and Mint oil extracts should not be used as inhibitors against the isolated bacteria.

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