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Antimicrobial activity of plant essential oils against the growth of Escherichia coli

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ABSTRACT:*Plant essential oils a well known herbs that was widely used in traditional medicine and food preservation due to the antimicrobial properties. The present study aimed to evaluate the antimicrobial activities of natural essential oils alone and in combination with tetracycline antibiotic against E.coli growth. In this study the antimicrobial effects of rosemary (Rosemarinus officinalis), clove(Sygium aromaticus), cumin(Cuminum cyminum) and ginger(Zingiber officinale) were tested alone and with tetracycline at concentration of (1:1, 1:2, 1:5 and 1:10) w/v dilution concentration which incorporated into Muller Hinton Agar that inoculated with pure activated E.coli culture and incubated for 24 hours at 37°C , the inhibition zone diameter was recorded. The obtained result showed that clove and rosemary essential oil alone was indicated the antimicrobial action, while clove, rosemary and ginger in combination with tetracycline were exhibited much more antimicrobial action than in tetracycline positive control against E.coli growth. This study emphasize the 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.*

KEY WORDS *:Antimicrobial activity, Esherichia coli, Inhibition zone, Plant essential oils, Tetracycline*

I. INTRODUCTION

Natural products have gained a special attention in the recent years because of increasing the phenomena of acquiring antibiotic resistance by different bacterial species (Al-Sheddi,2009). The use of plant essential oils in both the food and the pharmaceutical industries has been developed interestingly, a systematic examination of plant extracts for these properties has become increasingly important. The use of natural antimicrobial compounds is important not only in the preservation of food but also in the control of microbial growth in the diseases condition (Baratta et al., 1998, Nazia et al 2011 and Seenivasan et al 2006). Several species and herbs exert antibacterial influences due to their essential oil fractions. Some scientists revealed the antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic, and onion against both bacteria and molds. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity (Omidbeygi et al. 2007:YesilCeliktaset al. 2007).The essential oil extracted from the dried flower buds of cloves is used for acne, warts, scars and parasites. Research has shown that clove oil is used as a topical application to relieve pain and to promote healing and also finds use in the fragrance and flavoring industries (Chaieb et al., 2007). However, clove oil is toxic to human cells (Prashar etal., 2006). Essential oils derived from rosemary thyme and other herbs were shown to inhibit osteoclast activity and increase bone density in vitro (Putnamet al., 2006). The present study was therefore conducted to evaluate the antibacterial potential of essential oil of clove, Rosemary, Cumin and Ginger with different dilution concentrations against *Escherichia coli*.

II. MATERIAL AND METHODS

2,1. Microorganism and oils

Microorganism that was selected for this study is E. coli which was obtained from medical bacteriology laboratory of Biology Dept. in the college of science, Salahaddin University- Erbil. For inoculums preparation: Exactly 18 hour broth culture of the test bacteria isolates was suspended into sterile nutrient broth. They were standardized according to National Committee for Clinical Laboratory Standards (NCCLS) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0 × 106 cfu/ml.

The plant essential oils were purchased from (HEMANI company) in the local market and different dilution concentrations of the oils were prepared (1:1, 1:2, 1:5 and 1:10mg/ml).

2,2. Antimicrobial activity

A standard disc diffusion method by Baurer et al 1966 was used. In each experiment, microorganisms were cultured at 37 °C for 24 hours and prepared to turbidity equivalent to McFarland standard No. 0.5. Then100 µL of the suspension was spread on the test plate (Muller Hinton Agar). Sterile discs (6 mm diameter) were impregnated with 10 µL of the essential oils and placed on the surface of the test plate. Control discs were saturated with tetracycline (10 µg/disc).Plates were subsequently incubated at the appropriate temp for 24hrs and zones of inhibition were calculated by measuring the diameter in mm.

III. RESULT AND DISCUTION

The recorded data of antibacterial action of rosemary alone revealed that the significant maximum inhibition zone ($P<0.05$) was exhibited in the 1:5 dilution concentration with mean inhibition zone reached (11.5 mm) and standard error about (0.463), however this value was significantly less than the positive control which was treated with Tetracycline antibiotic ($P<0.05$). While the results of plant oil and antibiotic combination indicated the largest inhibition zone in the first (1:1) dilution concentration with mean value (33.38 mm) and standard error (1.24) which was significantly ($P<0.05$) more potent against *E. coli* growth than Tetracycline alone in positive control (Mean value was 25.37mm with standard error 0.498) respectively as shown in Table (1) and Figure (1).

Table (1): Susceptibility and inhibition zone diameter (mm) of different plant essential oils alone and in combination with tetracycline against *E.coli* growth.

Treatments	Rosemary		Clove		Cumin		Ginger	
	Mean	S E	Mean	S E	Mean	S E	Mean	S E
Control	25.3750	.49776	25.3750	.49776	25.3750	.49776	25.1429	.50843
1:1	7.6250	.18298	12.0000	1.13389	-ve	-ve	-ve	-ve
1:2	8.5000	.32733	8.5000	.56695	-ve	-ve	-ve	-ve
1:5	11.5000	.46291	7.7500	.41188	-ve	-ve	-ve	-ve
1:10	9.6667	.42164	7.8333	.60093	-ve	-ve	-ve	-ve
1:1 TC*	33.3750	1.23834	28.2500	.55902	23.6250	.41993	25.5714	.64944
1:2 TC	29.8750	.58056	28.3750	.41993	24.5000	.62678	24.5000	.42817
1:5 TC	29.3750	.41993	23.8750	.63913	25.2500	.52610	24.7143	.60609
1:10 TC	30.2500	.25000	25.2500	.41188	24.6250	.65295	26.2857	.52164

TC= Tetracycline, SE= Standard error

In our study, rosemary oil alone was exhibited intermediate action against *E.coli* growth according the standard ranges of inhibition zone diameter (Ravi et al., 2010). The antimicrobial activities of rosemary against *E.coli* and some other microorganisms was confirmed by (O. Al-Jiffri et al., 2011).The combination of rosemary with tetracycline exhibit the strong antimicrobial action against *E.coli* growth according the inhibition zone diameter standards (Ravi et al., 2010), this result was indicated that rosemary oil could be administered with tetracycline for treatment of *E.coli* infections and food preservations. In spite of our result,(O. Al-Jiffri et al., 2011) detected not significant anti *E.coli* action in such combination when compared with tetracycline alone. The major constituent of rosemary are alpha-pinene (24.1%),1,8-ceneole (23.5%) and camphor (19.7%),within these compounds camphor assumed to be a major attributor to the antimicrobial activities (Shigeharu et al., 2001).

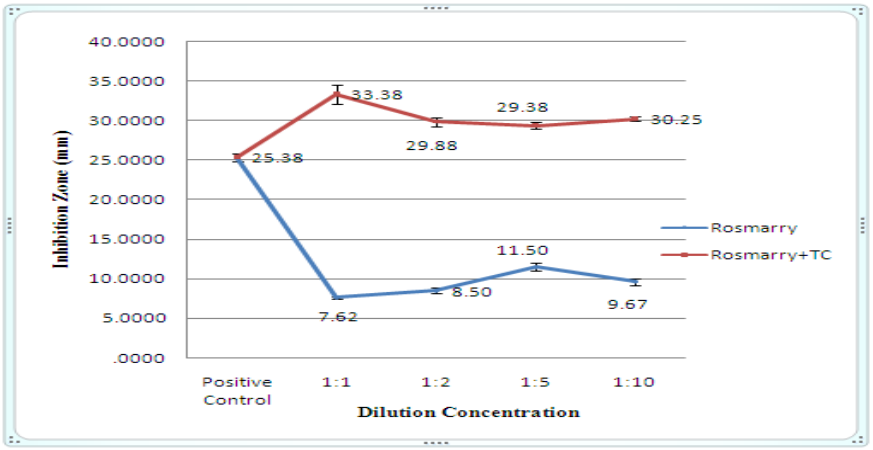

Figure (1) : Inhibition zone diameter (mm) in different dilution concentrations of rosemary alone and in combination with tetracycline against E.coli

Figure (1) : Inhibition zone diameter (mm) in different dilution concentrations of rosemary alone and in combination with tetracycline against E.coli

The synergistic potential of rosemary essential oil with tetracycline could be also considered as a sources of compounds that increase the sensitivity of bacterial cells to antibiotics.

The antimicrobial properties of clove oil alone and in combination with tetracycline against *E.coli* growth presented in table (1) and figure (2), the study showed the significant maximum inhibition zone diameter of clove oil alone (P<0.05) was exhibited in the first (1:1 w/v) dilution concentration with mean inhibition zone diameter about (12.00mm) and standard error (1.133)which was significantly less than the inhibition zone diameter of tetracycline positive control (P<0.05) was (mean value 25.37mm with standard error 0.497) as shown in table (1), while the inhibition zone diameter of clove in combination with tetracycline was in the second (1:2w/v) dilution concentration with mean value about (28.37mm) and standard error (0.491)was significantly (P<0.05) more effective against *E.coli* growth than both clove oil and tetracycline positive control antibiotic alone respectively.

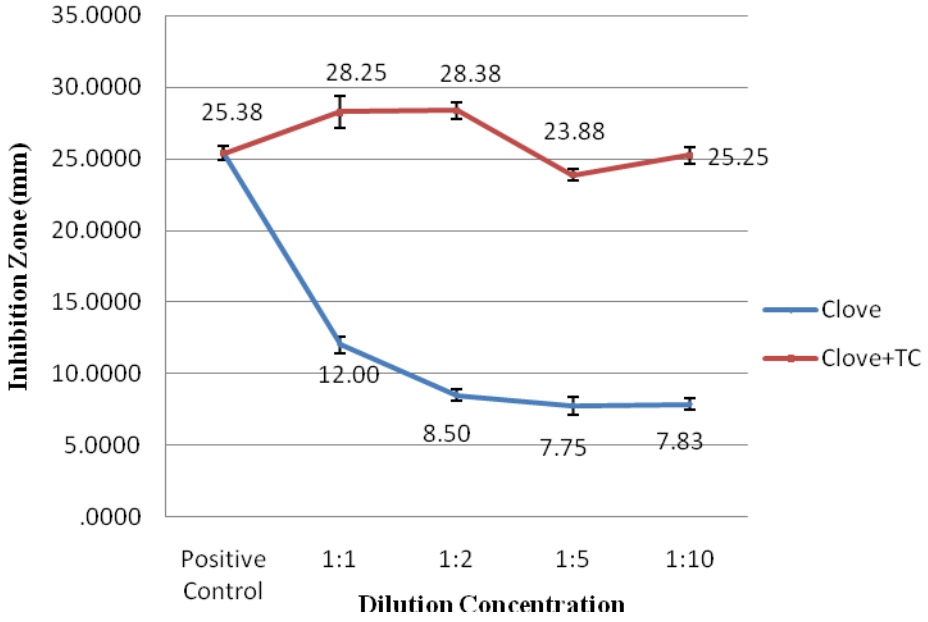

Figure (2) : Inhibition zone diameter (mm) in different dilution concentrations of clove alone and in combination with tetracycline against E.coli

` The antimicrobial properties of clove oil against *E.coli*, other gram negative and gram positive bacteria was confirmed by (Charu et al 2008), previous work (Bin Shan et al 2007) emphasized the importance of phenolic compounds and antioxidant capacity in the antimicrobial activity of clove extract. The present study indicated that clove oil alone exhibited the moderate antimicrobial activities against *E.coli* growth according the inhibition zone diameter if compared with the strong antimicrobial action of tetracycline positive control (Ravi et al., 2010). The antimicrobial activity of clove oil is attributed to eugenol (2-methoxy4-allyl phenol)and high tannin content which provides additional antimicrobial activities(Suree and Pana., 2005).The mode of antimicrobial actions related to these components produce ability to inactivate microbial adhesion, enzymes and cell envelope protein (Charu et al 2008). The obtained data was indicated the synergism between clove oil in combination with tetracycline antibiotic against *E.coli* growth (O. Al-Jiffri et al., 2011),their inhibition zone diameter was more than in both clove and tetracycline alone, that was showed the presence of some components mentioned above that enhance the affectivity of tetracycline against *E.coli* (Charu et al 2008).

The recorded data pertaining the antimicrobial potential of the cumin oil alone and in combination with tetracycline were presented in table (1) and figure (3), the result of the disc diffusion test of cumin oil alone against *E.coli* growth revealed no significant inhibition zone was observed around the bacterial colonies on the plate agar medium, while the antimicrobial action of cumin oil in combination with tetracycline was indicated the largest inhibition zone diameter in the third (1:5 w/v) dilution concentration with mean value about (25.25mm) and standard error (0.526) was no significantly ($P<0.05$) differences if compared with the antimicrobial action of tetracycline positive control (mean value 25.37 mm, with standard error 0.497).

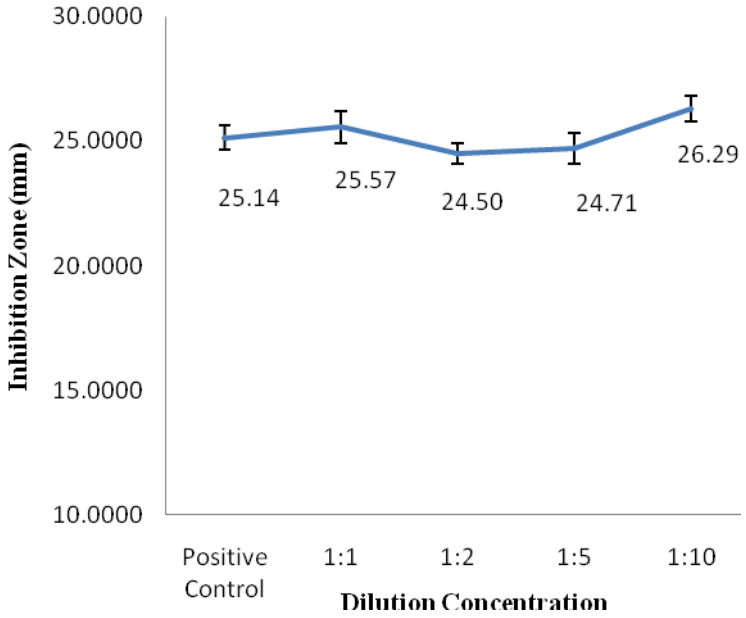

Figure (3) : Inhibition zone diameter (mm) in different dilution concentrations of cumin in combination with tetracycline against E.coli

Cumin oil alone doesn't exhibit any antimicrobial properties against *E.coli* growth, however previous studies confirmed that cumin oil possess the antimicrobial action against some species of gram negative and gram positive bacteria (Suree and Pana., 2005 and de Guzman., 1999). The antimicrobial property is attributed to the major constituents of cumin which are cuminaldehyde and monotrepenehydrocarbones(Suree and Pana., 2005).The combination mode of cumin essential oil with tetracycline indicated approximately equal degree of antimicrobial action in comparison with the tetracycline positive control, no synergism process was occur between them. Research results about the effect of four different dilution concentrations of ginger oil alone and in combination with tetracycline positive control were presented in table (1) and figure (4), result of the disc diffusion test of ginger oil alone against *E.coli* growth revealed no significant inhibition zone was observed around the bacterial colonies, while the antimicrobial action of ginger oil in combination with tetracycline was indicated the largest inhibition zone diameter in the (1:10 w/v) dilution concentration with mean value about (26.28mm) and standard error (0.521) was significantly more effective against *E.coli* growth than tetracycline (mean value 25.142 mm with standard error 0.508).

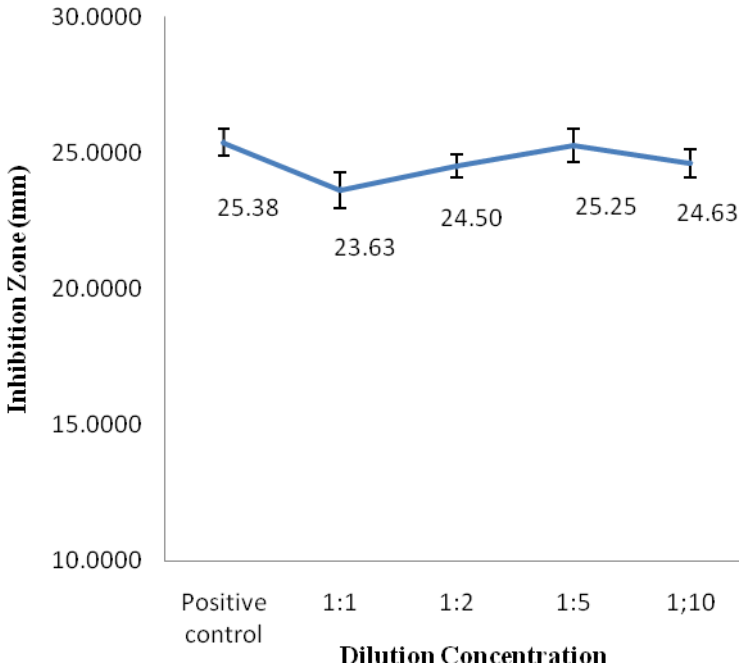

Figure (4) : Inhibition zone diameter (mm) in different dilution concentrations of ginger in combination with tetracycline against E.coli

In our study, ginger oil alone doesn't exhibit any antimicrobial properties against *E.coli* growth, however previous studies were confirmed that ginger oil possess the antimicrobial action in different degrees against some species of gram negative and gram positive bacteria depending on the species of bacteria (Suree and Pana., 2005, Indu and A. Narmala., 2010 and , Gao D, Zhang Y., 2010). The combination mode of ginger essential oil with tetracycline showed significantly more potent antimicrobial action than tetracycline positive control, herbal medicines are sometimes administered concomitantly with antibiotic (Estimone et al, 2006), this can lead to either beneficial or deleterious effects. The gingerol and shogaol are the major components of ginger, that could be responsible agents for the antimicrobial properties of ginger, they are phenolic compounds rupture the bacterial cell membrane and loss of their properties (Masniari., 2011). The synergism between ginger oil and tetracycline was verified by (O. Al-Jiffri et al., 2011), the ginger components that mentioned above may be attributable to increase the effectiveness of tetracycline when used in combination mode.

IV. CONCLUSION

In conclusion, the degree of antimicrobial properties of four spices tested alone against *E.coli* growth can be put in the following order: (clove >rosemary > ginger and cumin), while the degree of antimicrobial properties of four spices tested in combination with tetracycline against *E.coli* growth can be put in the following order: (rosemary > clove > ginger > cumin), clove and rosemary essential oils alone were exhibited a strong antibacterial action. These spices may be selected as potentially useful anti *E.coli* agents in meat products and other foods which easily contaminated by *E.coli* through fecal oral route during bad handlings, a possible way to use these two spices in combination with other food preservatives such as salt, acid, sugar and others to select pathogenic and spoilage microorganisms and may provide better alternatives in the conventional antimicrobial additives in food.

This study emphasize the antibacterial properties of rosemary, clove and ginger essential oils in combination with tetracycline against *E.coli*, it has been observed that the antibacterial affectivity of all the essential oils tested in combination with tetracycline much higher than that of each tetracycline and essential oils alone when tested *in vitro*, such combinations can be recommended for therapeutic purpose and be used as alternative medicine.

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