ARTICLE / INVESTIGACIÓN

The inhibitory effect of some plant essential oils on the growth of some bacterial species

Mohammed Abdullah Mahmood[1], Shaker Gazi Gergees[2], Alaa Taha Younis AL-Hammadi[1]*
DOI. 10.21931/RB/2023.08.01.52
[1] Biology Department, College of Education Pure Science, Mosul University, Mosul, Iraq.
[2] Biology Department, College of Science, Mosul University, Mosul, Iraq.
Corresponding author: alaatyounis@uomosul.edu.iq

Abstract: This research is aimed at investigating the inhibitory essential oils activity of thyme, clove, cinnamon, ginger and garlic plants against some positive and negative gram pathogenic bacteria (*Escherichia coli, proteus spp., Staphylococcus epidermidis, Staphylococcus aureus*) by using the disk diffusion method And compares this activity with the activity of standard antibiotics, then study the synergistic and antagonistic effect between these essential oils with the antibiotics used against these bacteria. The results represented the effectiveness of all essential oils on the studied bacteria to varying degrees, except the clove oil, which doesn't show any inhibitory effect on *E.coli* and *S.epidermidis*. In contrast, cinnamon oil had the most inhibiting impact on all the bacteria studied. While garlic and ginger oil showed the lowest Inhibitory, thyme oil showed a strong inhibitory effect on *E.coli* and a moderate effect on other studied bacteria. Also, the results showed the synergistic effect between most essential plant oils and most antibiotics against most bacterial strains. The antagonistic effect was between essential oils with a few studied antibiotics toward some bacterial strains.

Key words: Plant, essential oils, bacteria, the inhibitory effect.

Introduction

The essential oil has been used medically throughout history to treat various disease infections over a long period, such as skin treatment, burns, cancers and other diseases. The widespread interest in the use of essential oils has emerged in the modern with the emergence of the department of aromatherapy as an alternative medicine based on other compounds and oil that has shown good treatment effects, The emergence of bacterial resistance to conventional antibiotics and drugs have led other researchers to search for alternative therapeutic biocidal Sources against different pathogenic bacteria[1].

The antimicrobial activity of essential oils (Eos) has been known through research and tested in vitro towards a wide range of pathogens; these oils are secondary metabolites enriched in chemical compounds[2,3]. It has been used recently in food preservation and treatment for types of cancers[4-7]. The main components of (essential oils) are phenolics, alcoholics, hydrocarbons, aldehydes and ketones which are answerable for medicinal plant biological activities. The cell membrane of bacterial cells is the main target of the mechanism oils[8,9]. Medicinal plants and their oils were used in folk medicine. Naturally, essential oils play a significant role in producing plants against pathogens. The essential oils contain secondary metabolic substances that can inhibit and kill many bacteria, yeasts and molds. The bacterial cell membrane is the main target for making these oils and other targets in bacteria[10]. The inhibitory efficacy of essential oils against pathogenic pathogens has been investigated because of the emergence of multiple resistances to these pathogens to conventional antibiotics, which is a significant problem in treating many bacterial diseases[11].

People in various parts of the world have historically used ginger, thyme, clove, cinnamon and garlic for various purposes, so the purpose of this research is to examine in vitro behavior of the oils toward two Gram-negative bacterial strains and two Gram-positive bacterial strains and study a mixing of such essential oils and standard antibiotics in vitro action.

Materials and methods

Essential Oils

Five essential oils were collected from local markets equipped by the Itimad Company, Iraq (Table 1). Such oils have been selected based on survey literature and used for medical purposes.

Local name	Name Botanical
Thyme	*Thumus vulgaris*
Clove	*Syzygium aromaticum*
Cinnamon	*Cinnamomum Cassia*
Ginger oil	*Zingiberofficinableg*
Garlic oil	*Allium sativum*

Table 1. Essential oils used in this study.

Bacteria isolates

Two types of Gram-negative bacteria (*Escherichia coli, Proteus spp.*), and two types of Gram-positive bacteria (*Staphylococcus epidermidis, Staphylococcus aureus*). These isolates were obtained as pure identifiable isolates from the biology department/college of science/ Mosul University. The bacteria cultures were stored at 4 ° C in their appropriate agar slants and used as stock cultures throughout the study. Mueller Hinton agar (MHA).

Citation: Mahmood M A, Gergees S G, Younis AL-Hammadi A T. The inhibitory effect of some plant essential oils on the growth of some bacterial species. Revis Bionatura 2023;8 (1) 52. http://dx.doi.org/10.21931/RB/2023.08.01.52
Received: 23 December 2022 / **Accepted:** 30 January 2023 / **Published:** 15 March 2023

Antibiotics

Five disks of antibiotics were used-manufactured by *Bioanalyses* (Turkey). Amoxicillin Clavulanic acid(AMC 30µg), Ceftriaxone (CTX 30µg), Trimethoprim- Sulphamethoxazole (SXT 25µg), Gentamicin (CN 10µg) and Ciprofloxacin(CIP 10µg).

The Antibiotics sensitivity test

Antibiotics sensitivity test against studied bacterial isolates was conducted according to the modified method of Kirby-Bauer[12].

Antibacterial activity of Essential oils Test

The method of diffusion of discs was used in this research. An overnight culture has been calibrated to 0.5 McFarland requirements 10^8 CFU/ml for each microbial strain. Five hundred µL suspensions were spread out Mueller-Hinton agar (MHA) plates. With aseptic conditions, 10 µL of essential oil was impregnated with empty sterilized filter paper disks (6 mm diameter) of five types and placed on the surface of the agar. For 18-24h, the inoculated plaques were incubated at 37 ° C. Antimicrobial activity was measured against the test species by calculating the inhibition zone (mm). Every assay has been repeated 2 times[13].

Results and discussion

The Antibiotics sensitivity test

The results of the sensitivity of the (*E.coli, Proteus spp., S.aureus, and S.epidermidis*) to a group of antibiotics were shown in Table 2 and Figure 1 (A-D), where contrasts between these isolates can be noticed in the manner of their resistance to antibiotics.

The antiprogram of different isolates showed that all tested isolates were sensitive toward the selected standard antibiotics; the antibiotic sensitivity test was performed to compare the antibacterial activities of tested essential oils with the inhibitory action of antibiotics.

Antibacterial activity of Essential oils

Antibacterial activities of five "essential oils" toward four bacterial species are shown in Table 3.

These results showed that the oil of cinnamon exhibits superior antibacterial activity toward all bacteria, with inhibition zones (12 to 25 mm). This demonstrated the highest antibacterial activities in *E.coli* (25 mm), and the lowest inhibition effect observed in both *S.epidermidis* (12 mm), as shown in Figure 2 (A-D). Depending on the susceptibility of the bacteria studied, clove oil produced an inhibition zone toward *E.coli* and *S.aureus* only (12mm, 7mm), respectively, as shown in Figure 2 (A-D). In contrast, the location of inhibition produced by garlic and ginger essential oils varies from (8mm to 12mm). For garlic oil, the lowest inhibitory zone was observed against *S.epidermidis* (8mm), but the lowest inhibitory area was *E.coli* and *S.aureus* (12 mm), as shown in figures(B 1-4); for ginger oil, the lowest inhibitory zone was observed against *S.aureus* (8mm), but the top inhibitory zone was showed toward *E.coli*, and *S.epidermidis* (12mm) as shown in Figure 2 (A-D) and the inhibition zone of essential oil Thyme produced differs from (8mm to 20mm). The minimal inhibition zone was observed against *Proteus spp.* and *S.aureus* (8mm). In comparison, the optimum inhibition zone was for E.coli (20 mm) as shown in Figure 2 (A-D).

In vitro experiments in this study have shown that the essential oils of Ginger, Clove, Thyme, Cinnamon and Garlic show antibacterial activity toward the studied bacteria, but their activities varied. Many studies on the antimicrobial activity of plant compounds toward various types of microbes have been released, including food pathogthese[14] Clove and Thyme oils demonstrated solid antibacterial activity with inhibition zones at (8-20mm) and (7-12mm).

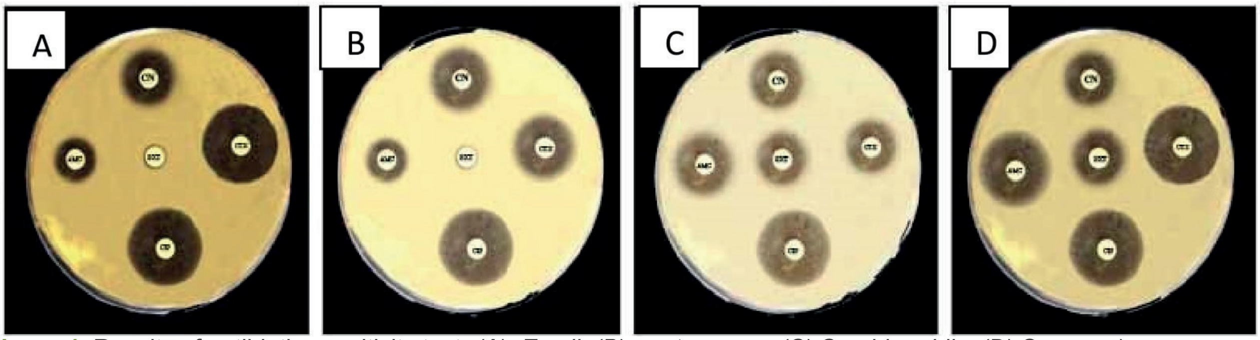

Figure 1. Results of antibiotic sensitivity test. *(A): E.coli; (B): proteus spp. (C) S.epidermidis; (D) S.aureus).*

Bacteria	CN (10) µg	SXT (25) µg	CTX(30)	CIP(10) µg	AMC(30) µg
E. coli	15	-	23	23	10
Proteus spp.	20	-	20	25	10
S.epidermidis	15	15	20	23	20
S.aureus	15	18	15	25	20

(-): NO Inhibitory effect

Table 2. Result of the standard antibiotics sensitivity test against the bacterial isolates, inhibition zones in mm.

Bacteria	Clove	Thyme	Cinnamon	Garlic	Ginger
E. coli	-	20	25	12	12
Proteus spp.	12	8	14	10	10
S.epidermidis	-	12	12	8	8
S.aureus	7	8	15	12	12

(-): NO Inhibitory effect

Table 3. Antibacterial activities of "essential oils" (10µl / disc) toward bacterial strains.

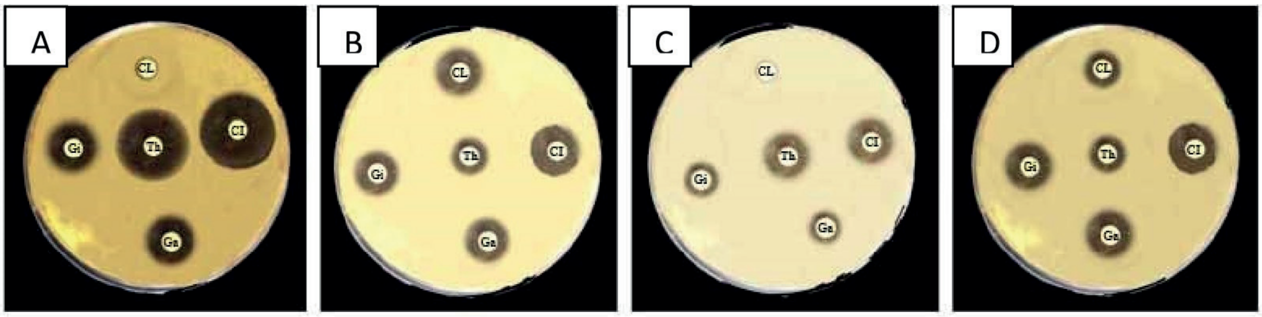

Figure 2. Antibacterial activity of five tested Essential oils. (Th-Thyme, Cl-Clove, Ci-Cinnamon, Gi-Ginger, Ga-Garlic). *(A): E.coli; (B): proteus spp. (C) S.epidermidis; (D): S.aureus.*

Accordingly, such results consent with[15] Clove oil is active against foodborne "Gram-positive" bacteria (*Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis and Listeria monocytogenes*). And "Gram-negative" bacteria (*P.aeruginosa, E.coli, Yersinia enterocolitica, Salmonella choleraesuis*). And Clove oil had the highest activity with a measured inhibition zone (23.7 mm) towards *V.cholerae*[16,17] the essential thyme oil was reported to have better activity against E.coli. Toward results were in agreement with the results of (18), which indicated that essential oil of thyme exhibited higher activity against *S.aureus* (20.1 mm), *B.subtilis*, and *S.aureus* (Gram-positive bacteria) and were much more sensitive than Gram-negative bacteria to the essential oils. The combined effect of the compounds results in the antimicrobial activities of essential oils.

The "antimicrobial activity" of essential oil of thyme and thymol was assessed in other tests, like (19), where the substantial activity of thyme toward *E.coli* and *Salmonella spp.* has been documented. An essential thyme oil antimicrobial effect was also recorded toward *E.coli* and other foodborne bacteria[20].

The cinnamon essential oil has shown the best antibacterial activity toward whole bacterial species studied; these results are by (21), in which cinnamon oil exhibited potent activity against bacterial strains selected. And the other side, multiple studies showed that cinnamon and clove oils have precise and reliable effects on different pathogens and bacteria[22].

Several studies have shown that oils' activity can be attributed to their hydrophobicity as a feature of "essential oils" and their components, which allow them to separate bacterial cell membrane lipids, challenge the cell well structures and make them more permeable[23]. And the exit of critical ions and molecules will lead to death[24].

Aromatogram test for combinations of essential oils with antibiotics

Applying Cinnamon oil with all standard antibiotics tested, the antimicrobial activity of all antibiotics increased against the four bacterial strains tested, as shown in Table 4 and Figures 3 (A-D).

The application of thyme with all standard antibiotics tested led to enhance the antibacterial activities of these antibiotics, except the combination with (AMC and SXT) resulted in a decrease in the inhibition zone exerted by the antibiotics when used alone against *S.epidermidis* as shown in Table 5 and Figures 4 (A-D). Toroglu said combining basic Thyme oil and standard in vitro antibiotics resulted in an

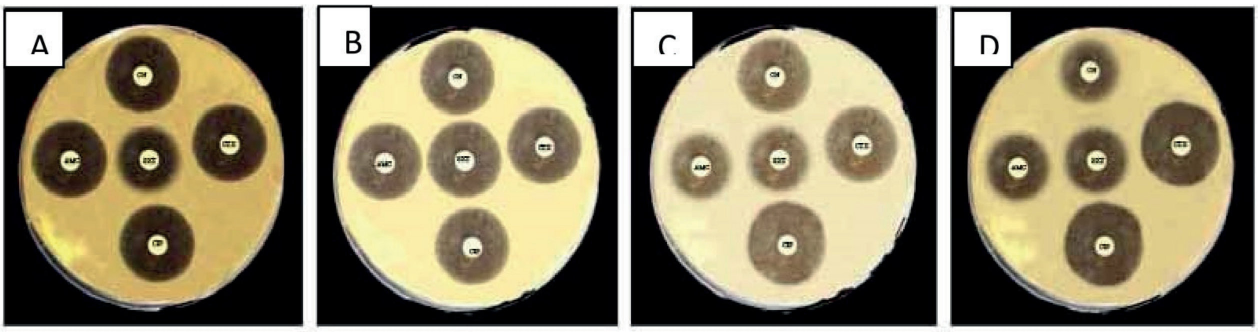

Figure 3. (A): Inhibitory activity of Combination between cinnamon oil with tested antibiotics against *E.coli*. (B): Inhibitory activity of Combination between cinnamon oil with tested antibiotics against *proteus spp*. (C): Inhibitory activity of Combination between cinnamon oil with tested antibiotics against *S.epidermidis*. (D): Inhibitory activity of Combination between cinnamon oil with tested antibiotics against *S.aureus*.

Bacteria	Essential oil	Standard antibiotic discs					Standard antibiotic discs with essential oil				
		CN	SXT	CTX	CIP	AMC	CN	SXT	CTX	CIP	AMC
E. coli	25	15	-	23	23	10	25	20	25	25	25
Proteus spp.	14	20	-	20	25	10	25	25	25	25	25
S.epidermidis	12	15	15	20	23	20	25	22	25	30	20
S.aureus	15	15	18	15	25	20	18	20	30	30	20

(-): NO Inhibitory effect

Table 4. The antibacterial activities (inhibition zones) of Cinnamon essential oil and its synergistic with antibiotic effects.

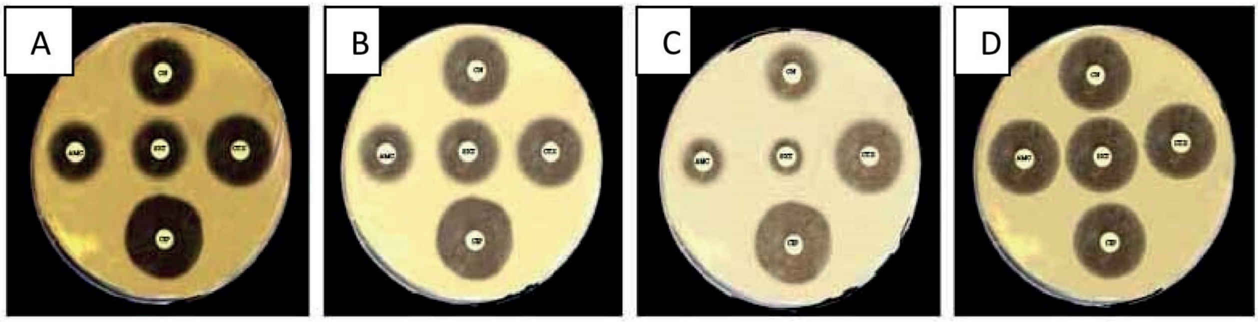

Figure 4. (A) Inhibitory activity of Combination between Thyme oil with tested antibiotics against *E.coli*. (B) Inhibitory activity of Combination between Thyme oil with tested antibiotics against *proteus spp*. (C): Inhibitory activity of Combination between Thyme oil with tested antibiotics against *S.epidermidis.* (D): Inhibitory activity of Combination between Thyme oil with tested antibiotics against *S.aureus.*

Bacteria	Essential oil	Standard antibiotic discs					Standard antibiotic discs with essential oil				
		CN	SXT	CTX	CIP	AMC	CN	SXT	CTX	CIP	AMC
E. coli	20	15	-	23	23	10	20	17	23	30	15
Proteus spp.	8	20	-	20	25	10	22	20	22	30	15
S.epidermidis	12	15	15	20	23	20	15	9	25	30	12
S.aureus	8	15	18	15	25	20	25	25	25	25	25

(-): NO Inhibitory effect

Table 5. The antimicrobial activities (inhibition zones) of Thyme essential oil and its synergistic with antibiotic effects. Mm

antagonistic effect on bacteria[25].

Applying Ginger oil with all tested standard antibiotics led to the antagonistic effect of these antibiotics against all bacteria tested except the combination of (SXT) against *E. coli, Proteus spp.* , and (CTX) against *S.epidermidis* , *S.aureus* . And (AMC) against *Proteus spp.*, and (CN) against *S.aureus*, which led to a synergistic effect as shown in Table 6 Figure 5 (A-D).

The combination of Garlic oil with all tested standard antibiotics resulted in a synergistic effect against all tested bacteria except the case of applying this oil with (CXT) against *E.coli, Proteus spp.*, which led to a decrease in the antimicrobial activity of this antibiotic compared when used as alone, also antagonistic effect occurred with (SXT) against the *S.epidermidis* as shown in Table 7 Figure 6 (A-D).

Applying Clove oil with all tested standard antibiotics resulted in a synergistic effect with most antibiotics against most tested bacteria except in the case of applying this oil with (SXT, AMC) against *S.epidermidis* only, as shown in Table 8 and Figure 7 (A-D).

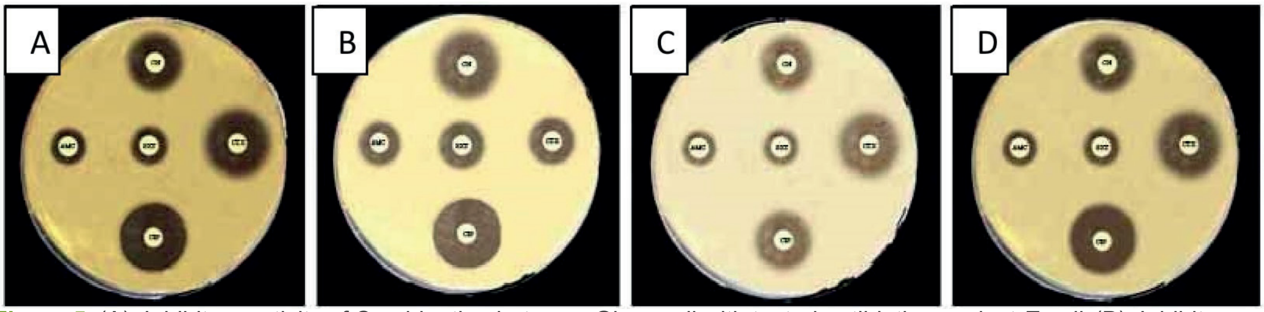

Figure 5. (A): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *E.coli.* (B): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *proteus spp*. (C): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *S.epidermidis.* (D): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *S.aureus.*

Bacteria	Essential oil	Standard antibiotic discs					Standard antibiotic discs with essential oil				
		CN	SXT	CTX	CIP	AMC	CN	SXT	CTX	CIP	AMC
E. coli	12	15	-	23	23	10	14	8	18	20	7
Proteus spp.	10	20	-	20	25	10	16	11	11	22	10
S.epidermidis	12	15	15	20	23	20	13	8	20	16	9
S.aureus	8	15	18	15	25	20	16	9	18	23	9

(-): NO Inhibitory effect

Table 6. The antimicrobial activities (inhibition zones) of ginger essential oil and its synergistic with antibiotic effects. Mm

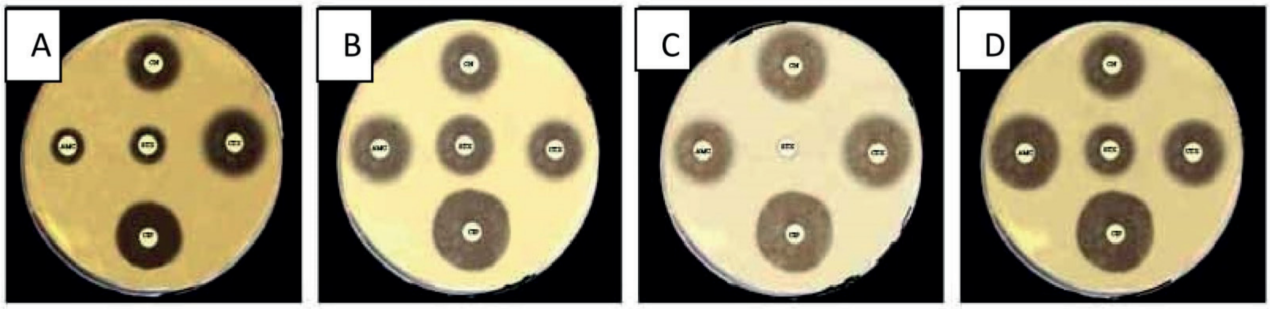

Figure 6. (A) Inhibitory activity of Combination between Garlic oil with tested antibiotics against *E.coli.* (B) Inhibitory activity of Combination between Garlic oil with tested antibiotics against *proteus spp.* (C) Inhibitory activity of Combination between Garlic oil with tested antibiotics against *S.epidermidis*. (D)Inhibitory activity of Combination between Garlic oil with tested antibiotics against *S.aureus.*

Bacteria	Essential oil	Standard antibiotic discs					Standard antibiotic discs with essential oil				
		CN	SXT	CTX	CIP	AMC	CN	SXT	CTX	CIP	AMC
E. coli	12	15	-	23	23	10	15	8	18	25	10
Proteus spp.	10	20	-	20	25	10	22	21	15	28	18
S.epidermidis	8	15	15	20	23	20	26	-	26	30	23
S.aureus	12	15	18	15	25	20	18	15	18	27	25

Table 7. The antimicrobial activities (inhibition zones) of essential garlic oil and its synergistic with antibiotic effects. Mm

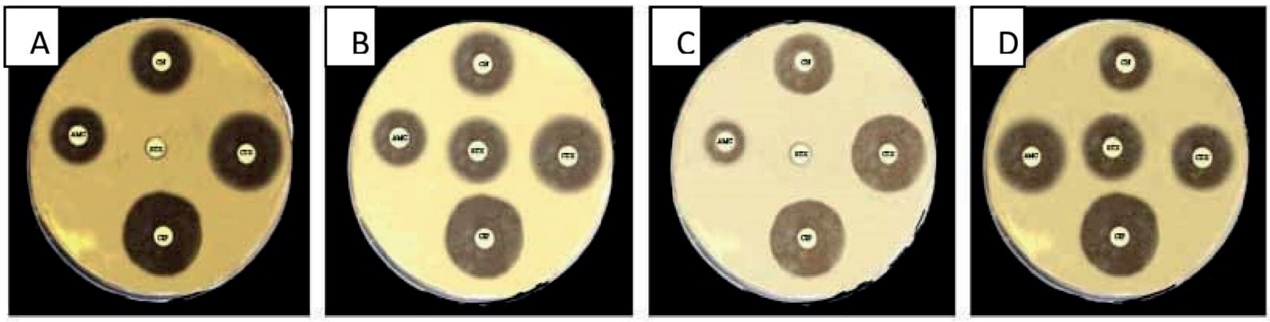

Figure 7. (A): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *E.coli*. (B): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *proteus spp*. (C): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *S.epidermidis*. (D): Inhibitory activity of Combination between Ginger oil with tested antibiotics against *S.aureus*.

Bacteria	Essential oil	Standard antibiotic discs					Standard antibiotic discs with essential oil				
		CN	SXT	CTX	CIP	AMC	CN	SXT	CTX	CIP	AMC
E. coli	-	15	-	23	23	10	20	-	25	30	15
Proteus spp.	12	20	-	20	25	10	22	22	23	30	15
S.epidermidis	-	15	15	20	23	20	16	-	24	23	11
S.aureus	7	15	18	15	25	20	15	20	20	30	25

Table 8. The antimicrobial activities (inhibition zones) of Clove essential oil and its synergistic with antibiotic effects. Mm

Conclusions

The study shows that combining "essential oils" from these medicinal plants with traditional antibiotics has considerable potential for producing and eliminating new antimicrobial treatments for many microorganism-caused diseases. Based on the results obtained, the essential oils function with the standard antibiotics studied. This collaboration could result in new treatment options for infectious diseases and increasing drug resistance. More molecular-based studies of synergistic interaction are required to understand the synergistic mechanism, which is fundamental to gggggggg pharmacological agents for treating bacterial infections using medicinal plants. The research will also focus on finding medicinal plants with synergistic activity in that direction.

Acknowledgments

The authors are very grateful to the University of Mosul / College of Education Pure Science / Biology Department for their provided facilitates, which helped to improve the quality of this work.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Author Contributions

All authors contributed equally to this work.

Funding

This is self-funding research.

Ethics Statement

Not applicable.

Bibliographic references

1. Abad. M, J, Ansuategui., M and Bermejo., P.(2007) .Active antifungal substances from natural sources.ARCHIVOC; 2007, 116–145.
2. Kalemba., D, and Kunicka. A. (2003) .Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, 10: 813–829.
3. Al-Bayati, F. (2009). Isolation and identification of antimicrobial compound from menthe longifolia l, leaves grown wild in Iraq. Annals of clinical microbiology and antimicrobials 8:20.
4. Faid., M, Bakhy., K, Anchad., M, Tantaoui-Elaraki., AandAlomondpaste.,A.(1995).Physicochemical and microbiological characterizations and preservation with sorbic acid and cinnamon Food Prod 1995; 58:547-550.
5. Buttner., M,P, Willeke., K, Grinshpun., S,A.(1996) Sampling and analysis of airborne microorganisms. In Manual of Environmental Microbiology Edited by: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV. ASM Press: Washington, DC: 629-640.
6. Van de Braak., S, A, A, J and Leijten. G,C,J,J. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam. 1999: 116.
7. Sylvestre., M, Pichette.,A, Longtin., A, Nagau.,F.andLegault., J.(2006).Essential oil analysis and anticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe.J Ethnopharmacol; 103:99-102.
8. Di Pasqua., R, Betts., G, Hoskins., N, Edwards., M, Ercolini., D andMauriello., G. (2007).Membrane toxicity of antimicrobial compounds from essential oils.Journal of Agricultural and Food Chemistry; 55, 4863–4870.
9. Mahmood, M. A., & Essa, M. A. (2021). Antimicrobial activity of peptides extracted from camels' blood neutrophils against some pathogenic bacteria. Iraqi Journal of Veterinary Sciences, 35(1), 33-37.
10. Nazzaro., F.; Fratianni. F, De Martino., L, Coppola R and De Feo. , V (2013). Effect of Essential Oils on Pathogenic Bacteria, Pharmaceuticals, 6;1451-1474
11. Andradea.,B, F ,M,Barbosaa., L ,N,Probsta I da &Júniora.,A. F .(2014)Journal of Essential Oil Research 26:1, 34-40 .
12. Clinical and Laboratory Standards Institute/National Comitee for clinical Laboratory Standards (CLSI/ NCCLS). (2012). In: Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Information Supplement. CLSI/NCCLS document M 100–S15. Wayne, PA,
13. Oulkheir .,S, Aghrouch.,M , El Mourabit.,F, Dalha.,F, Graich.,H , Amouch.,F, Ouzaid.,K, Moukale., A and S. Chadli.(2017). Antibacterial Activity of Essential Oils Extracts from Cinnamon, Thyme, Clove and Geranium Against a Gram Negative and Gram Positive Pathogenic Bacteria. Journal of Diseases and Medicinal Plants; 3(2-1): 1-5
14. Friedman., M, Henika., R,P, Mandrell., E,R.(2002).Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica.J. Food Protect. 65:1545-1560.
15. Lopez., P, Sanchez. Batlle., R, Nerin., C .(2005). Solid- and Vapor-phase antimicrobial activities of six essential oils: susceptibility of selected food borne bacterial and fungal strains. J. Agric. Food Chem., 53 (17): 6939-6946.
16. Sabahat., S and Perween., T. (2008). In vitro antibacterial activity of Clove against Gram negative bacterial Pak. J. Bot.; 40(5): 2157-2160.
17. Deise., F.S, Francielli., P,K.J, Régis., A, Z, Karine., B, S, Andressa., F and Leadir., L,M, F.(2014).Antimicrobial Activity of the Essential Oil of Thyme and of Thymol against Escherichia coli Strains.Acta Scientiae Veterinariae.; 42: 1234
18. Bosnić., T, Softić., D, and Grujić-Vasić. J, (2006). Antimicrobial Activity of Some Essential Oils and Major Constituents of Essential Oils.Acta MedicaAcademica ;35:19-22 .
19. Ivanovic., J, Misic., D, Zizovic., I and Ristic., M.(2012) In vitro control of multiplication of some food-associated bacteria by thyme, rosemary and sage isolates. Food Control. 25(1): 110116.
20. Silva N, Alves S, Gonçalves A, Amaral JS, Poeta P. (2013). Antimicrobial activity of essential oils from Mediterranean aromatic plants against several foodborne and spoilage bacteria. Food Science and Technology International. 19(6): 503-510.
21. Rabuseenivasan., S, Jayakumar. M and Ignacimuthu.,S. (2006) In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine, 6:39.
22. Matan., N, Rimkeeree., H, Mawson., A, J, Chompreeda., P, Haruthaithanasan., V and Parker M.(2006) Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. Into J Food Microbial; 107:180-185.
23. Sikkema, J., de Bont, J.A.M. and Poolman, B. (1994) Interactions of Cyclic Hydrocarbons with Biological Membranes. Journal of Biological Chemistry, 269, 8022-8028.
24. Denyer., S.P (1995) Mechanisms of action of antibacterial biocides international-biodeterioration-and-biodegradation Volume 36 Issues 3–4 Pages 195-475
25. Toroglu.,S.(2007). In vitro antimicrobial activity and antagonistic effect of essential oils from plant species.J.Environ Biol.;28:551-9