JBAAR

Journal of Bioscience and Applied Research

https://jbaar.journals.ekb.eg

SPBH

Efficacy of *Nigella sativa* and *Zingiber officinale* Extract Against Multidrug-Resistance Escherichia *coli:* An Experimental Study

Mohammed Aladeeb[1], Ahmed Al Qattan[2], Alaa Younis Mahdy Al-Hamadany[2], Ali M. Saadi[3], Zinah Ahmed Mohammed[2]

[1]Department of Radiology Techniques, Mosul Medical Technical Institute, Northern Technical University, Mosul, Iraq.

[2]Department of Anesthesia Techniques, Mosul Medical Technical Institute, Northern Technical University.

[3]Department of Medicinal Plant Technologies, Technical Agricultural College, Northern Technical University, Iraq

[*]**Corresponding author: E-mail address: ali.mohammed@ntu.edu.iq**

DOI:10.21608/jbaar.2024.329699.1094

Abstract

The effectiveness of a plant extract of *Nigella sativa* (black seed) and *Zingiber officinale* (ginger) against multidrug-resistant *Escherichia coli* Strains was carried out. Ten strains were selected from a previous study and diagnosed by biochemical tests and confirmed by the VITEK 2 device. Susceptibility testing against (10) selected antibiotics was performed, which proved that all strains were multidrug-resistant. The Agar Diffusion method was used to test the effectiveness of the extracts through discs saturated with methanol and water extracts at concentrations of 100 mg/ml.

Some strains showed non-significant inhibition zones around the discs which were saturated with methanolic extract. In contrast, the aqueous extracts did not show any noticeable effect. The Minimum Inhibitory Concentration was determined by the dilution method, where the ethanolic extracts reduced the turbidity of some strains at concentrations of (100 mg/ml). Still, the bacterial growth continued as scattered colonies when the samples were re-cultured on an agar medium. These results indicate that the plant extracts that were used did not achieve any effectiveness in inhibiting the growth of multidrug-resistant *Escherichia coli*, highlighting the need for further studies to explore other methods or concentrations.

Key words: *E. Coli*, Nigella sativa, Zingiber officinale, MIC

Introduction

When microorganisms can resist a different group of antimicrobials, this condition is known as Antibiotic Resistance. In such a state of resistance, microbes can develop this form of drug resistance [1]. Multidrug resistance is the ability of bacteria to resist multiple drugs. This type of bacteria has different resistance mechanisms, such as natural microbial resistance to some microbes, acquired microbial resistance, or genetic mutation [2,3]. Irrational use of antibiotics is causing a rise in antibiotic resistance, leading to high rates of deaths associated with antimicrobial resistance worldwide [4,5]. The commensal E. coli bacteria do not affect the host, but they can develop resistance mechanisms by acquiring resistance genes from other bacteria that can transform from commensal bacteria to multi-resistant bacteria [6,7]. The environment and host greatly influence the genetic structure of Escherichia coli strains, allowing bacteria to acquire diverse antibiotic resistance mechanisms [8,9]. To address these problems, we must rely on an alternative system by relying on

Received: September 23, 2024. Accepted: November 9, 2024. Published: November 23, 2024

natural materials, especially medicinal plants, and herbs, through intensive studies in developing materials that work as antibiotics or using modern therapeutic alternatives by developing some molecules of these materials to work as antibiotics against these infectious germs [6,10] Manufactured drugs are widely available and are used to eliminate bacteria that cause infectious diseases, but they also cause many side effects to patients. An example of such cases is meningitis, which is treated with the antibiotic chloramphenicol. This medication can induce aplastic anemia by bridging the blood-brain barrier [11] Humans have used this medicinal plant since ancient times, to eliminate a wide range of diseases and for other necessary purposes. Medicinal plants have long been used as a source of treatment in all societies. In addition, many traditional medicines contribute to modern medicine [12,13]. Many plants and spices are widely recognized for their antibacterial and antioxidant properties, and essential oils and extracts from these plants are rich in bioactive substances like phenolic compounds [14,15]. For centuries, the annual herbaceous *Nigella sativa* (NS) plant, a member of the buttercup family, has been widely used in traditional medicine [16]. This plant is widely found in India and also in the Middle East and North Africa. As a galactagogic and emmenagogue, it is beneficial for treating various ailments, including fever, eczema, cough, and asthma [17,18]. *Nigella sativa* exhibited strong antibacterial properties Against both Gram-negative and Gram-positive bacteria, as well as antibiotic-resistant strains, according to *in vitro* Studies published between the years 2000 and 2015. For example, Nigella sativa impeded the growth of bacteria such as *E. coli, Salmonella, and Helicobacter pylori*. which causes serious gastrointestinal morbidity. A literature review showed that *Nigella sativa* has strong antibacterial qualities against MDR strains *in vitro* [19] While ginger is known by the scientific name Zingiber Officinale, it is a plant from the family known as Zingiberaceae [20,21]. ginger is associated with the Indian subcontinent's tropical rainforest regions, but it has also been linked to countries in Southern Asia with minor differences in *Z. officinale*'s genetic composition [21,22]. One of those medicinal plants that has been very helpful in treating a variety of illnesses is ginger extract. Strong antimicrobial effects of ginger include killing or inhibiting the growth of pathogenic bacteria [21] anti-inflammatory and antioxidant (19) anti-thrombotic [24]. hypoglycemic [25], And antitumor [26].

This experimental study aims to evaluate the effectiveness of aqueous and ethanolic plant extracts of *Nigella sativa* and *Zingiber officinale* in inhibiting the growth of multi-resistant *Escherichia coli* isolates. The study focuses on testing the effectiveness of these extracts as potential natural alternatives to address the increasing challenges of bacterial infections due to the resistance of many bacterial strains to conventional antibiotics.

Methods:

Preparation of Plant Extracts:

The plant extracts (black seed and ginger) were extracted using two methods: aqueous extraction and solvent extraction.

1. Aqueous extraction method: Black seed seeds and fresh ginger roots were washed and at room temperature (25°C) they were dried and then crushed to a fine powder. 50 g of each powder was heated to boiling in 500 ml of distilled water for 30 min. After cooling the mixture, it was filtered using filter paper and then concentrated by evaporation to obtain a concentrated extract. Aqueous extracts are stored in sterile, opaque bottles at 4 °C for a while until use [27,28].
2. Solvent extraction method: *Nigella sativa* seeds and fresh ginger roots were washed and dried at room temperature, crushed into powder, add 50 grams of extract powder to 500 ml of 70% ethanol, leave the mixture to soak at room temperature for 48 hours with periodic stirring, then filter the extract mixture using filter paper, the extract is concentrated using an evaporating flask at 40-50 °C to form a dry extract, it is stored in opaque and sterile bottles at 4 °C until use [27,28].

Figure 1: Extraction of *Nigella sativa and Zingiber officinale* using ethanol solvent.

Isolation and identification of Multidrug-resistant *Escherichia coli*:

Multidrug-resistant *Escherichia coli* strains were selected from a previous study of urine samples of hemodialysis patients. The identity of these isolates was confirmed by biochemical tests and VITEK 2 device to accurately identify the bacterial strains [29,30].

Antibiotic Sensitivity Test:

The susceptibility of the selected bacterial strains to antibiotics was determined using the Kirby-Bauer method. Standard antibiotic discs were distributed on Mueller-Hinton agar and incubated for 24 h at 37°C. The inhibition zones around the discs were determined using a sterile ruler and the results were read according to Clinical Laboratory Standards Institute (CLSI) guidelines.

Disk Diffusion Test for plant extracts:

Plates containing Mueller-Hinton agar medium were inoculated with multidrug-resistant strains. Sterile paper discs were prepared and immersed in the plant extract at different concentrations (50, 100, 200) mg/ml The discs were distributed on the agar medium; the plates were incubated for 24 h at 37°C, and the diameters of the inhibition zones around the discs were determined to evaluate the antibacterial activity of each extract [31].

Minimum Inhibitory Concentration (MIC):

The Minimum Inhibitory Concentration (MIC) of plant extract was tested using the serial dilution method. Ethanol and aqueous extracts *Nigella sativa and Zingiber officinale* were prepared at an initial concentration of 100mg/ml. Serial dilutions were made at a ratio of 2:1 to reach the following concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56) mg/ml. 1 ml from each concentration was added to 9 ml of broth nutrient medium, and then 100 µl of a standard bacterial suspension was added for each strain of E. coli. The tubes were incubated for 24 h at 37°C, and bacterial growth was assessed visually based on turbidity to determine the minimum concentrations that inhibit bacterial growth [32].

Results:

1. Results of Isolation and Identification

Ten multidrug-resistant *Escherichia coli* were isolated from urine samples. These strains were

subjected to initial biochemical tests, such as oxidase and catalase, urease, and IMViC tests, in addition to sugar fermentation to detect the metabolic characteristics of *Escherichia coli*. After that, all strains were confirmed using the VITEK 2 device, which showed an exact match with *Escherichia coli* in all samples. This indicates that the strains have the genetic and physiological characteristics of multidrug-resistant *Escherichia coli*.

1. Antibiotic susceptibility testing

The susceptibility of *Escherichia coli* strains to 11 types of antibiotics was tested using the Kirby Bauer plate diffusion method. The inhibition result was determined and interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The results were as follows:

2. Disk Diffusion Test (Kirby-Bauer Test):

The effectiveness of plant extracts From *Nigella sativa* (black seed) and Z*ingiber officinale* (ginger) against Multidrug-resistant *Escherichia coli* strains were tested by the agar diffusion method with disks saturated with ethanolic and aqueous extracts prepared at a concentration of 100mg/ml. Although some strains showed inhibition zones around the discs saturated with ethanolic extracts, non-significant zones were obtained against *E. coli* strains. Based on these results, the plant extracts of Nigella Sative and Zingiber officinale e cannot be considered effective in inhibiting the growth of multidrug-resistant *Escherichia coli*.

3. Minimum Inhibitory Concentration for Plant Extracts:

Minimum inhibitory concentrations (MIC) of plant extracts of *Nigella sativa* and ginger against multiresistant *E. coli* strains were determined using the serial dilution method. Concentrations starting from 100mg/ml were used and the results showed that ethanol extracts reduced the turbidity in some strains at concentrations of 100mg/ml, which suggests a relative decrease in bacterial growth density. However, after re-culture of the diluted solution on a nutrient medium (agar), scattered bacterial colonies appeared, indicating that the ethanolic extracts did not completely inhibit the bacterial growth, but only reduced the bacterial density. As for the aqueous extracts, they did not show any effect on turbidity or colony growth, which reinforces their ineffectiveness in inhibiting the growth of multidrug-resistant *E. coli*. Based on these results, no minimum effective inhibitory concentration was recorded that could be relied upon to completely stop the growth of bacterial strains (*E. coli*).

Table 1: Number of Resistance and sensitivity strains of *Escherichia coli* against 11 antibiotics

Antibiotic(symbol)	Inhibition zones (mm)	Number of sensitive strains	Number of resistant strains
Amikacin (AK)	16-22	2	8
Cefepime (CFM)	0-6	1	9
Ciprofloxacin (CIP)	10-14	3	7
Doxycycline (DO)	8-12	1	9
Imipenem (IPE)	18-24	8	2
Nalidixic acid (NA)	0-5	2	8
Nitrofurantoin (F)	14-18	4	6
Novobiocin (NV)	0-3	1	9
Norfloxacin (NOR)	10-13	3	7
Tetracycline (TE)	8-12	2	8

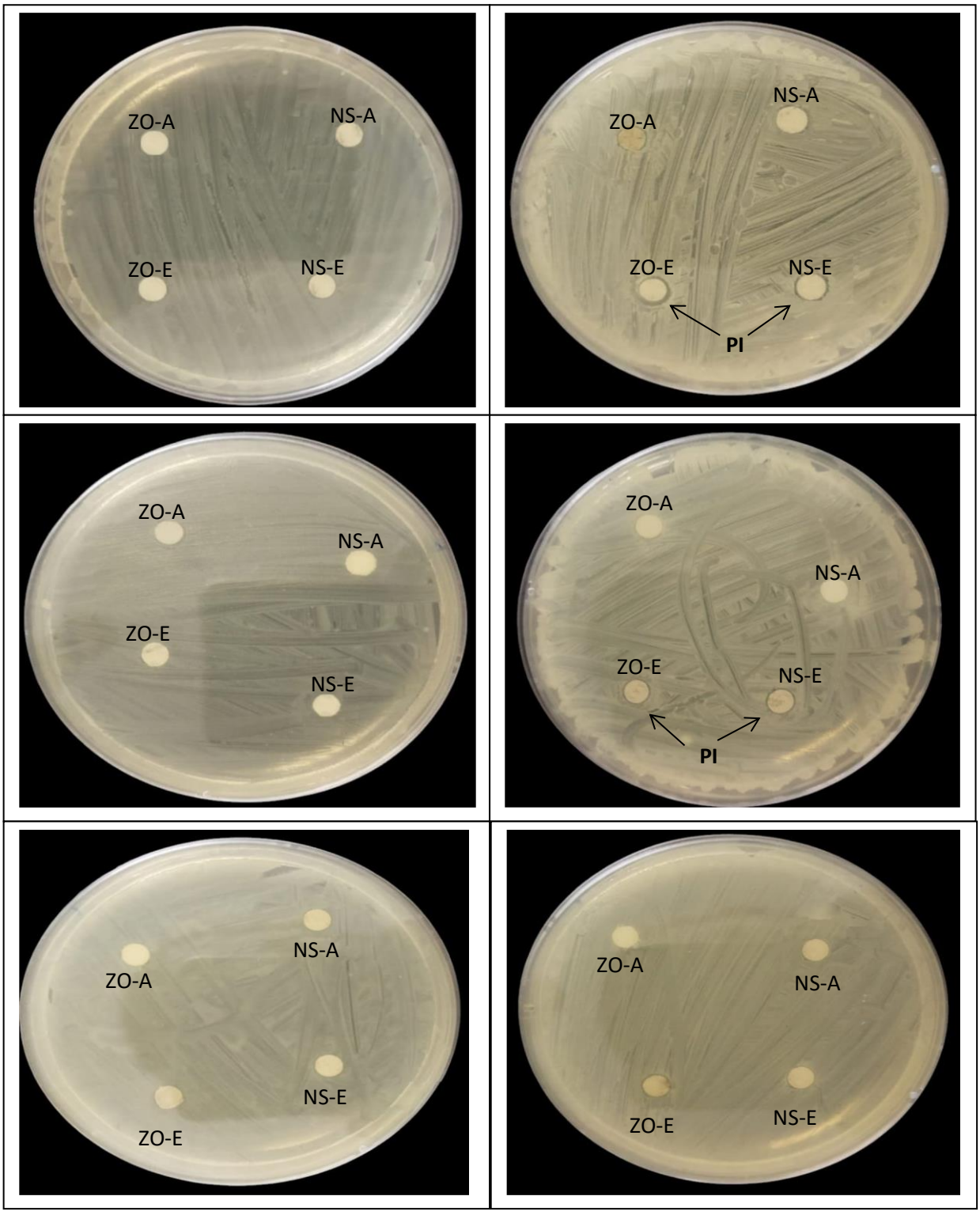

Figure 2: Disc diffusion test with aqueous and ethanolic extracts

ZO-A = Aqueous extract of Zingiber officinal, **ZO-E** = ethanol extract of Zingiber officinal
NS-A = Aqueous extract of Nigella sativa, **NS-E** = ethanol extract of Nigella sativa, **PI** = Poor Inhibition

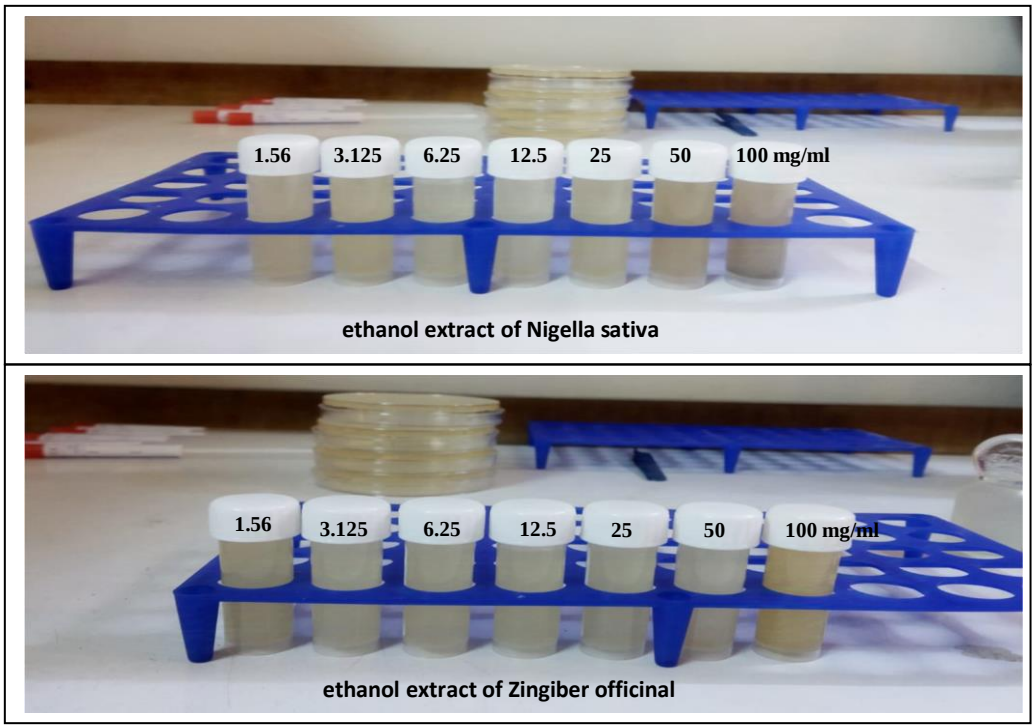

Figure 3: Minimum Inhibitory Concentration for Plant Extracts

Discussion

During this study, the effect of black seed extract (*Nigella sativa*) and ginger extract (Zingiber officinale) was studied. against multidrug-resistant *Escherichia coli* strains was evaluated. The results indicated that all strains were highly resistant, even when using ethanolic extracts, where small and statistically insignificant inhibition zones were observed. This is consistent with previous studies such as study [33] which indicated the resistance of bacterial strains against *Nigella sativa* extracts. Another study in Jordan found no inhibitory effect of isolates using Nigella sativa aqueous extract [34].

As for Zingiber officinale, the strains showed similar resistance, as observed in the study [35]. A study also showed a weak effect of *Zingiber officinale* against *E. coli* compared to other extracts such as garlic [36]. In contrast, a study conducted in Baghdad, Iraq, by Duaa Yassin and colleagues showed that high concentrations of up to 1000 mg/ml were effective in inhibiting the bacteria of *Escherichia coli* [37]. Another study supported these findings, as the same concentration of 1000 mg/ml was required to achieve an inhibitory effect [38,39]. However, a study conducted in Malaysia showed that concentrations of 100 mg/ml of *Zingiber officinale* extract had no significant effect on E. coli, while it was effective against *S. aureus and P. aeruginosa* [40,41].

These results suggest that *E. coli* has evolved multiple resistance mechanisms, including the use of multiple efflux pumps, which contribute significantly to its drug resistance [42,43]. Other reasons include structural differences such as unique cell envelope composition [44]. Biofilms are also one of the most complex causes of resistance to antimicrobial agents [45]. Furthermore, resistance occurs naturally through point mutations that result in changes in amino acids [46]. In addition, some plant extracts are only effective at high concentrations, where they must reach a sufficient

concentration at the target site of the bacteria to show their antibacterial effect [47,48].

Based on these results, it appears that the *Nigella sativa and Zingiber officinale* extracts were not sufficient in this study to effectively inhibit multidrug-resistant *E. coli*. However, this should not be considered a weakness of the study, but rather evidence of the complexity of bacterial resistance.

Conclusion

This study indicates that the effect of *Nigella sativa* and *Zingiber officinale* off extracts, whether ethanolic or aqueous extract at a concentration of 100 mg/ml, was not sufficiently effective inhibitory against multidrug-resistant E. coli isolates. Although some slight responses were shown with ethanolic extracts of some isolates. Accordingly, further research is required on different extraction methods and increasing the concentrations used, in addition to studying other plant compounds that may be more effective against multidrug-resistant *Escherichia coli*.

Conflict of interest: None

Funding: None

Reference

1. Colson, A. R., Morton, A., Årdal, C., Chalkidou, K., Davies, S. C., Garrison, L. P., ... & Xiao, Y. (2021). Antimicrobial resistance: is health technology assessment part of the solution or part of the problem?. Value in Health, 24(12): 1828-1834. https://doi.org/10.1016/j.jval.2021.06.002.
2. Catalano, A., Iacopetta, D., Ceramella, J., Scumaci, D., Giuzio, F., Saturnino, C., ... & Sinicropi, M. S. (2022). Multidrug resistance (MDR): A widespread phenomenon in pharmacological therapies. Molecules, 27(3): 616. https://doi.org/10.3390/molecules27030616.
3. Almola, H. (2022). Molecular Study of Acinetobacter baumannii that Lacking Some Essentials Genes Responsible for Toxin-Antitoxin System. *Archives of Razi Institute*, *77*(1): 483.
4. Karuniawati, H., Hassali, M. A. A., Suryawati, S., Ismail, W. I., Taufik, T., & Hossain, M. S. (2021). Assessment of knowledge, attitude, and practice of antibiotic use among the population of Boyolali, Indonesia: a cross-sectional study. International journal of environmental research and public health, 18(16); 8258. https://doi.org/10.3390/ijerph18168258
5. Al-Layla, E. A. M., AlTaie, A. A., Haddad, M. F., Abdullah, B. A., & Saadi, A. M. (2024). Molecular Detection of Asymptomatic Bacteriuria and its Bacteriophage from Adolescents in Mosul City/Iraq. *Journal of Bioscience and Applied Research*, *10*(3): 504-517.
6. Szmolka, A., & Nagy, B. (2013). Multidrug-resistant commensal Escherichia coli in animals and its impact on public health. Frontiers in Microbiology, 4(7): 258. https://doi.org/10.3389/fmicb.2013.00258.
7. Jawad, A., Kadhim, A., Hashim, M. Prevalence of multi-drug resistant Staphylococcus aureus and Escherichia Coli isolated from urinary tract. *Journal of Medical and Life Science*, 2024; 6(3): 410-419. doi: 10.21608/jmals.2024.383094
8. Taillon, O., Skurnik, D., Picard, B. & Denamur, E. (2010). The population genetics of commensal *Escherichia coli.* Nature Reviews Microbiology, 8(3): 207-217. https://doi. org/10.1038/nrmicro2298.
9. Shaker, E. M., Younis, T. I., Al-Hussein, R. Y. H. & Saadi, A. M. (2024). Knowledge, Attitude, and Practices of Community regarding Solid Waste Management (SWM). *Journal of Bioscience and Applied Research*, *10*(3): 318-327.
10. Torres, M. D. T. & de la Fuente-Nunez, C. (2019). Reprogramming biological peptides to combat infectious diseases. Chemical

Communications, 55(100): 15020-15032. https://doi.org/10.1039/C9CC07898C.

11. Jeon, S. J., Oh, M., Yeo, W. S., Galvao, K. N. & Jeong, K. C. (2014). The underlying mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases. PLoS One, 9(3): e92723. https://doi.org/10.1371/journal.pone.0092723.
12. Mohsen, S., Dickinson, J. A. & Somayaji, R. (2020). Update on the adverse effects of antimicrobial therapies in community practice. Canadian Family Physician, 66(9): 651-659.
13. Almola, A. H., Mahdy Alhamadany, A. Y., Haddad, M. F., & Sultan, S. M. (2021). Assessment of Genotoxic Effect Of Escherichia Coli In Patients With Urinary Tract Infection. *Biochemical & Cellular Archives*, *21*.
14. Dar, R. A., Shahnawaz, M. & Qazi, P. H. (2017). General overview of medicinal plants: A review. The journal of phytopharmacology, 6(6), 349-351.
15. Majhenič, L., Škerget, M., & Knez, Ž. (2007). Antioxidant and antimicrobial activity of guarana seed extracts. Food Chemistry, 104(3), 1258-1268. https://doi.org/10.1016/j.foodchem.2007.01.074 .
16. Sahoo, S. U. P. R. A. V. A., Singh, S. H. I. K. H. A., & Nayak, S. A. N. G. H. A. M. I. T. R. A. (2014). Chemical composition, antioxidant and antimicrobial activity of essential oil and extract of *Alpinia malaccensis* Roscoe (Zingiberaceae). International Journal of Pharmacy and Pharmaceutical Sciences, 6(7):183-188.
17. Zuridah, H., Fairuz, A. R. M., Zakri, A. H. Z. & Rahim, M. N. A. (2008). In vitro antibacterial activity of Nigella sativa against Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and *Bacillus cereus*. Asian Journal of Plant Sciences, 7(3:) 331-333.
18. Mahmoud and, H., Sepahvand, A., Jahanbakhsh, S., Ezatpour, B., & Mousavi, S. A. (2014). Evaluation of antifungal activities of the essential oil and various extracts of Nigella sativa and its main component, thymoquinone against pathogenic dermatophyte strains. Journal de mycologie medicale, 24(4): e155-e161. https://doi.org/10.1016/j.mycmed.2014.06.048.
19. Bakal, S. N., Bereswill, S., & Heimesaat, M. M. (2017). Finding novel antibiotic substances from medicinal plants—antimicrobial properties of Nigella sativa directed against multidrug resistant bacteria. European Journal of Microbiology and Immunology, 7(1), 92-98. https://doi.org/10.1556/1886.2017.00001.
20. Dilis, V., & Trichopoulou, A. (2010). Antioxidant intakes and food sources in Greek adults. the Journal of Nutrition, 140(7), 1274-1279. https://doi.org/10.3945/jn.110.121848.
21. Irzoqy, M. E., Sultan, S. J., Abdallah, O. D., Sultan, S. M., Abdullah, Y. J., & Saadi, A. M. (2024). Inhibitory Effect of Saccharomyces cerevisiae Against Propionibacterium acnes. *Journal of Bioscience and Applied Research*, *10*(3), 385-391.
22. Elghareeb, O., El-sherbiny, H., Nisa, N., Abou-El-magd, R. Evaluation of the ameliorative role of ginger (Zingiber officinale) extract against carbon tetrachloride-induced hepatotoxicity in male albino rats. *Journal of Medical and Life Science*, 2023; 5(4): 296-307. doi: 10.21608/jmals.2023.326295
23. Everett, T. H. (1980). The New York botanical garden illustrated encyclopedia of horticulture (Vol. 10). Taylor & Francis.
24. Pakyz, A., Powell, J. P., Harpe, S. E., Johnson, C., Edmond, M., & Polk, R. E. (2008). Diversity of antimicrobial use and resistance in 42 hospitals in the United States. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 28(7), 906-912. https://doi.org/10.1592/phco.28.7.906.
25. Murugesan, S., Venkateswaran, M. R., Jayabal, S., & Periyasamy, S. (2020). Evaluation of the antioxidant and anti-arthritic potential of

Zingiber officinale e Rosc. by in vitro and in silico analysis. South African Journal of Botany, 130, 45-53. https://doi.org/10.1016/j.sajb.2019.12.019.

26. Thomson, M., Al-Qattan, K. K., Al-Sawan, S. M., Alnaqeeb, M. A., Khan, I., & Ali, M. (2002). The use of ginger (Zingiber officinale e Rosc.) as a potential anti-inflammatory and antithrombotic agent. Prostaglandins, leukotrienes and essential fatty acids, 67(6), 475-478. https://doi.org/10.1054/plef.2002.0441.
27. Ojewole, J. A. (2006). Analgesic, antiinflammatory and hypoglycaemic effects of ethanol extract of Zingiber officinale e (Roscoe) rhizomes (Zingiberaceae) in mice and rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 20(9), 764-772. https://doi.org/10.1002/ptr.1952.
28. Liao, D. W., Cheng, C., Liu, J. P., Zhao, L. Y., Huang, D. C., & Chen, G. T. (2020). Characterization and antitumor activities of polysaccharides obtained from ginger (Zingiber officinale e) by different extraction methods. International journal of biological macromolecules, 152, 894-903. https://doi.org/10.1016/j.ijbiomac.2020.02.325.
29. Kozłowska, M., Ścibisz, I., Przybył, J. L., Laudy, A. E., Majewska, E., Tarnowska, K., ... & Ziarno, M. (2022). Antioxidant and antibacterial activity of extracts from selected plant material. Applied Sciences, 12(19), 9871. https://doi.org/10.3390/app12199871.
30. Silva, E., Fernandes, S., Bacelar, E., & Sampaio, A. (2016). Antimicrobial activity of aqueous, ethanolic and methanolic leaf extracts from Acacia spp. and Eucalyptus nicholii. African journal of traditional, complementary and alternative medicines, 13(6), 130-134. https://doi.org/10.21010/ajtcam.v13i6.18.
31. Granato,P.A.; Morton,V.; Morello,J.A.(2019). Laboratory Manual and Work Book in Microbiology Applications to Patients Care. MaGrow-Hill Education, New York, USA.
32. Wanger, A., Chavez, V., Huang, R., Wahed, A., Dasgupta, A., & Actor, J. K. (2017). Microbiology and molecular diagnosis in pathology: a comprehensive review for board preparation, certification and clinical practice.
33. Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology.
34. Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 48(suppl_1), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5.
35. Maryam, A. J., Fatimah, A. A., Ebtesam, A. K., Abdulrahman, A. S., & Ineta, B. E. (2016). In-vitro studies on the effect of Nigella sativa Linn., seed oil extract on Multidrug Resistant Gram positive and Gram negative bacteria. Journal of Medicinal Plants, 4(2), 195-199.
36. Abu-Al-Basalc, M. A. (2009). In vitro and in vivo anti-microbial effects of Nigella sativa Linn. seed extracts against clinical isolates from skin wound infections. American Journal of Applied Sciences, 6(8), 1440-1447. https://doi.org/10.3844/ajassp.2009.1440.1447.
37. Chand, B. (2013). Antibacterial effect of garlic (Allium sativum) and ginger (Zingiber officinale e) against Staphylococcus aureus, Salmonella typhi, Escherichia coli and Bacillus cereus. Journal of microbiology, biotechnology and food sciences, 2(4), 2481-2491.
38. Gull, I., Saeed, M., Shaukat, H., Aslam, S. M., Samra, Z. Q., & Athar, A. M. (2012). Inhibitory effect of Allium sativum and Zingiber officinale e extracts on clinically important drug-resistant pathogenic bacteria. Annals of Clinical Microbiology and Antimicrobials, 11, Article 8. https://doi.org/10.1186/1476-0711-11-8.
39. Al-Hamadany, A. Y. M., Mahmood, A. T., Khalaf, S. D., & Saadi, A. M. (2024). Evaluation

of genotoxicity and some biochemical parameters in preeclampsia patients with SARS-Cov2. *Journal of Bioscience and Applied Research*, *10*(3), 352-361.

40. Yassen, D., & Ibrahim, A. E. (2016). Antibacterial activity of crude extracts of ginger (Zingiber officinale e Roscoe) on Escherichia coli and Staphylococcus aureus: A Study in vitro. Indo American Journal of Pharmaceutical Research, 6(06), 5830-35.
41. Sultan, S. M., Saady, A. M., & Irzoqy, M. E. (2018). A Comparative Study of the Effect of Alcoholic Extract of Turmeric Plant in Inhibiting the Growth of Candida Albicans. *International Journal of Engineering and Technology*, *7*(4.37), 12-16.
42. Othman, A. S., Azizan, A., Shambely, S., & Mohamad, A. (2020). Antibacterial activity of Zingiber officinale e against foodborne pathogens. International Journal of Psychosocial Rehabilitation, 24(07), 9666-9673.
43. Jafar, N. B., Fadhil, Z. H., Mustafa, E. M., & Saadi, A. M. (2022). Investigation the inhibitory effects of AgNPs generated by Bifidobacterium spp. on bacteria isolated from ready-to-eat foods. *Food Science and Technology*, *42*, e88721.
44. Zainal, A. A., Salleh, N. F. M., Ahmad, W. A. N., Rasudin, N. S., Zaabar, W. R. W., & Ghafar, N. A. (2022). Antioxidant properties and antimicrobial effect of Zingiber officinale e extract towards Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. IOP Conference Series: Earth and Environmental Science, 1102, 012049. https://doi.org/10.1088/1755-1315/1102/1/012049.
45. Teelucksingh, T., Thompson, L. K., & Cox, G. (2020). The evolutionary conservation of Escherichia coli drug efflux pumps supports physiological functions. Journal of Bacteriology, 202(22), 10-1128. DOI: https://doi.org/10.1128/jb.00367-20.
46. Cloete, T. E. (2003). Resistance mechanisms of bacteria to antimicrobial compounds. International Biodeterioration & Biodegradation, 51(4), 277-282. https://doi.org/10.1016/S0964-8305(03)00042-8.
47. Ito, A., Taniuchi, A., May, T., Kawata, K., & Okabe, S. (2009). Increased antibiotic resistance of Escherichia coli in mature biofilms. Applied and environmental microbiology, 75(12), 4093-4100. DOI: https://doi.org/10.1128/AEM.02949-08.
48. Hopkins, K. L., Davies, R. H., & Threlfall, E. J. (2005). Mechanisms of quinolone resistance in Escherichia coli and Salmonella: recent developments. International journal of antimicrobial agents, 25(5), 358-373. https://doi.org/10.1016/j.ijantimicag.2005.02.006.